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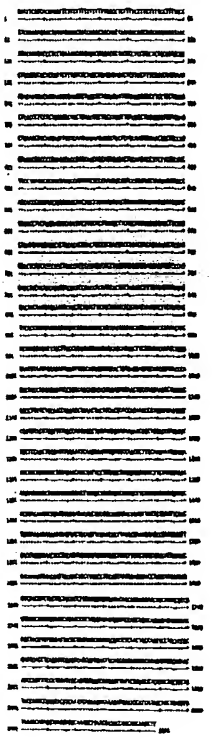
(54) Title: NUCLEOTIDE AND PROTEIN SEQUENCES OF VERTEBRATE DELTA GENES AND METHODS BASED THEREON

(57) Abstract

The present invention relates to nucleotide sequences of vertebrate *Delta* genes, and amino acid sequences of their encoded proteins, as well as derivatives (e.g., fragments) and analogs thereof. In a specific embodiment, the vertebrate Delta protein is a human protein. The invention further relates to fragments (and derivatives and analogs thereof) of Delta which comprise one or more domains of the Delta protein, including but not limited to the intracellular domain, extracellular domain, DSL domain, domain amino-terminal to the DSL domain, transmembrane region, or one or more EGF-like repeats of a Delta protein, or any combination of the foregoing. Antobidies to Delta, its derivatives and analogs, are additionally provided. Methods of production of the Delta proteins, derivatives and analogs, e.g., by recombinant means, are also provided. Therapeutic and diagnostic methods and pharmaceutical compositions are provided. In specific examples, isolated Delta genes, from *Xenopus*, chick, mouse, and human, are provided.



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NUCLEOTIDE AND PROTEIN SEQUENCES OF VERTEBRATE DELTA GENES AND METHODS BASED THEREON

This application claims priority to United States Provisional Application Serial No. 60/000,589 filed June 28, 1995, which is incorporated by reference herein in its entirety.

1. INTRODUCTION

The present invention relates to vertebrate *Delta* genes and their encoded protein products, as well as derivatives and analogs thereof. Production of vertebrate Delta proteins, derivatives, and antibodies is also provided. The invention further relates to therapeutic compositions and methods of diagnosis and therapy.

2. BACKGROUND OF THE INVENTION

Genetic analyses in Drosophila have been extremely useful in dissecting the complexity of developmental pathways and identifying interacting loci. However, understanding the precise nature of the processes that underlie genetic interactions requires a knowledge of the protein products of the genes in question.

The vertebrate central nervous system is an intimate mixture of different cell types, almost all generated from the same source - the neurogenic epithelium that forms the neural plate and subsequently the neural tube. What are the mechanisms that control neurogenesis in this sheet of cells, directing some to become neurons while others remain non-neuronal? The answer is virtually unknown for vertebrates, but many of the cellular interactions and genes controlling cell fate decisions during neurogenesis have been well characterized in *Drosophila* (Campos-Ortega, 1993, J. Neurobiol. 24:1305-1327). Although the gross anatomical context of neurogenesis appears very different in insects and vertebrates, the possibility remains that, at a cellular level, similar events are occurring via conserved molecular

mechanisms. Embryological, genetic and molecular evidence indicates that the early steps of ectodermal differentiation in *Drosophila* depend on cell interactions (Doe and Goodman, 1985, Dev. Biol. 111:206-219; Technau and Campos-Ortega, 1986, Dev. Biol. 195:445-454; Vässin et al., 1985, J. Neurogenet. 2:291-308; de la Concha et al., 1988, Genetics 118:499-508; Xu et al., 1990, Genes Dev. 4:464-475; Artavanis-Tsakonas, 1988, Trends Genet. 4:95-100). Mutational analyses reveal a small group of zygotically-acting genes, the so called neurogenic loci, which affect the choice of ectodermal cells between epidermal and neural pathways (Poulson, 1937, Proc. Natl. Acad. Sci. 23:133-137; Lehmann et al., 1983, Wilhelm Roux's Arch. Dev. Biol. 192:62-74; Jürgens et al., 1984, Wilhelm Roux's Arch. Dev. Biol. 193:283-295; Wieschaus et al., 1984, Wilhelm Roux's Arch. Dev. Biol. 193:296-307; Nüsslein-Volhard et al., 1984, Wilhelm Roux's Arch. Dev. Biol. 193:267-282). Null mutations in any one of the zygotic neurogenic loci -- *Notch* (*N*), *Delta* (*Dl*), *mastermind* (*mam*), *Enhancer of Split* (*E(spl)*, *neuralized* (*neu*), and *big brain* (*bib*) -- result in hypertrophy of the nervous system at the expense of ventral and lateral epidermal structures. This effect is due to the misrouting of epidermal precursor cells into a neuronal pathway, and implies that neurogenic gene function is necessary to divert cells within the neurogenic region from a neuronal fate to an epithelial fate.

Neural precursors arise in the *Drosophila* embryo from a neurogenic epithelium during successive waves of neurogenesis (Campos-Ortega & Hartenstein, 1985, The embryonic development of Drosophila melanogaster (Springer-Verlag, Berlin; New York); Doe, 1992, Development 116:855-863). The pattern of production of these cells is largely determined by the activity of the proneural and neurogenic genes. Proneural genes predispose clusters of cells to a neural fate (reviewed in Skeath & Carroll, 1994, Faseb J. 8:714-21), but only a subset of cells in a cluster become neural precursors. This restriction is due to the

action of the neurogenic genes, which mediate lateral inhibition - a type of inhibitory cell signaling by which a cell committed to a neural fate forces its neighbors either to remain uncommitted or to enter a non-neural pathway (Artavanis-Tsakonas & Simpson, 1991, Trends Genet. 7:403-408; Doe & Goodman, 1985, Dev. Biol. 111:206-219). Mutations leading to a failure of lateral inhibition cause an overproduction of neurons - the "neurogenic" phenotype (Lehmann et al., 1981, Roux's Arch. Dev. Biol. 190:226-229; Lehmann et al., Roux's Arch. Dev. Biol. 192:62-74). In *Drosophila*, the inhibitory signal is delivered by a transmembrane protein encoded by the *Delta* neurogenic gene, which is displayed by the nascent neural cells (Heitzler & Simpson, 1991, Cell 64:1083-1092). Neighboring cells express a transmembrane receptor protein, encoded by the neurogenic gene *Notch* (Fortini & Artavanis-Tsakonas, 1993, Cell 75:1245-1247). *Delta* has been identified as a genetic unit capable of interacting with the *Notch* locus (Xu et al., 1990, Genes Dev. 4:464-475).

Mutational analyses also reveal that the action of the neurogenic genes is pleiotropic and is not limited solely to embryogenesis. For example, ommatidial, bristle and wing formation, which are known also to depend upon cell interactions, are affected by neurogenic mutations (Morgan et al., 1925, Bibliogr. Genet. 2:1-226; Welshons, 1956, Dros. Inf. Serv. 30:157-158; Preiss et al., 1988, EMBO J. 7:3917-3927; Shellenbarger and Mohler, 1978, Dev. Biol. 62:432-446; Technau and Campos-Ortega, 1986, Wilhelm Roux's Dev. Biol. 195:445-454; Tomlison and Ready, 1987, Dev. Biol. 120:366-376; Cagan and Ready, 1989, Genes Dev. 3:1099-1112). Neurogenic genes are also required for normal development of the muscles, gut, excretory and reproductive systems of the fly (Muskavitch, 1994, Dev. Biol. 166:415-430).

Both Notch and Delta are transmembrane proteins that span the membrane a single time (Wharton et al., 1985, Cell 43:567-581; Kidd and Young, 1986, Mol. Cell. Biol. 6:3094-3108; Vässin, et al., 1987, EMBO J. 6:3431-3440;

Kopczynski, et al., 1988, Genes Dev. 2:1723-1735) and include multiple tandem EGF-like repeats in their extracellular domains (Muskavitch, 1994, Dev. Biol. 166:415-430). The *Notch* gene encodes a ~300 kd protein (we use "Notch" to denote this protein) with a large N-terminal extracellular domain that includes 36 epidermal growth factor (EGF)-like tandem repeats followed by three other cysteine-rich repeats, designated *Notch/lin*-12 repeats (Wharton, et al., 1985, Cell 43:567-581; Kidd and Young, 1986, Mol. Cell. Biol. 6:3094-3108; Yochem, et al., 1988, Nature 335:547-550). Molecular studies have lead to the suggestion that Notch and Delta constitute biochemically interacting elements of a cell communication mechanism involved in early developmental decisions (Fehon et al., 1990, Cell 61:523-534). Homologs are found in *Caenorhabditis elegans*, where the *Notch*-related gene *lin-12* and the *Delta*-related gene *lag-2* are also responsible for lateral inhibition (Sternberg, 1993, Current Biol. 3:763-765; Henderson et al., 1994, Development 120:2913-2924; Greenwald, 1994, Curr. Opin. Genet. Dev. 4:556-562). In vertebrates, several *Notch* homologs have also been identified (Kopan & Weintraub, 1993, J. Cell Biol. 121:631-641; Lardelli et al., 1994, Mech. Dev. 46:123-136; Lardelli & Lendahl, 1993, Exp. Cell Res. 204:364-372; Weinmaster et al., 1991, Development 113:199-205; Weinmaster et al., 1992, Development 116:931-941; Coffman et al., 1990, Science 249:1438-1441; Bierkamp & Campos-Ortega, 1993, Mech. Dev. 43:87-100), and they are expressed in many tissues and at many stages of development. Loss of *Notch-1* leads to somite defects and embryonic death in mice (Swiatek et al., 1994, Genes Dev. 8:707-719; Conlon et al., Rossant, J. Development (J. Dev. 121:1533-1545), while constitutively active mutant forms of *Notch-1* appear to inhibit cell differentiation in *Xenopus* and in cultured mammalian cells (Coffman et al., 1993, Cell 73:659-671; Kopan et al., 1994, Development 120:2385-2396; Nye et al., 1994, Development 120:2421-2430).

The EGF-like motif has been found in a variety of proteins, including those involved in the blood clotting cascade (Furie and Furie, 1988, Cell 53: 505-518). In particular, this motif has been found in extracellular proteins such as the blood clotting factors IX and X (Rees et al., 1988, EMBO J. 7:2053-2061; Furie and Furie, 1988, Cell 53: 505-518), in other *Drosophila* genes (Knust et al., 1987 EMBO J. 761-766; Rothberg et al., 1988, Cell 55:1047-1059), and in some cell-surface receptor proteins, such as thrombomodulin (Suzuki et al., 1987, EMBO J. 6:1891-1897) and LDL receptor (Sudhof et al., 1985, Science 228:815-822). A protein binding site has been mapped to the EGF repeat domain in thrombomodulin and urokinase (Kurosawa et al., 1988, J. Biol. Chem 263:5993-5996; Appella et al., 1987, J. Biol. Chem. 262:4437-4440).

Citation of references hereinabove shall not be construed as an admission that such references are prior art to the present invention.

3. SUMMARY OF THE INVENTION

The present invention relates to nucleotide sequences of vertebrate *Delta* genes (chick and mouse *Delta*, and related genes of other species), and amino acid sequences of their encoded proteins, as well as derivatives (*e.g.*, fragments) and analogs thereof. Nucleic acids hybridizable to or complementary to the foregoing nucleotide sequences are also provided. In a specific embodiment, the Delta protein is a mammalian protein, preferably a human protein.

The invention relates to vertebrate Delta derivatives and analogs of the invention which are functionally active, i.e., they are capable of displaying one or more known functional activities associated with a full-length (wild-type) Delta protein. Such functional activities include but are not limited to antigenicity [ability to bind (or compete with Delta for binding) to an anti-Delta antibody], immunogenicity (ability to generate antibody which binds to Delta), ability to bind (or compete with Delta for

binding) to Notch or other toporythmic proteins or fragments thereof ("adhesiveness"), ability to bind (or compete with Delta for binding) to a receptor for Delta. "Toporythmic proteins" as used herein, refers to the protein products of *Notch*, *Delta*, *Serrate*, *Enhancer of split*, and *Deltex*, as well as other members of this interacting set of genes which may be identified, *e.g.*, by virtue of the ability of their gene sequences to hybridize, or their homology to Delta, Serrate, or Notch, or the ability of their genes to display phenotypic interactions or the ability of their protein products to interact biochemically.

The invention further relates to fragments (and derivatives and analogs thereof) of a vertebrate Delta that comprise one or more domains of the Delta protein, including but not limited to the intracellular domain, extracellular domain, transmembrane domain, DSL domain, domain amino-terminal to the DSL domain, or one or more EGF-like (homologous) repeats of a Delta protein, or any combination of the foregoing.

Antibodies to a vertebrate Delta, its derivatives and analogs, are additionally provided.

Methods of production of the vertebrate Delta proteins, derivatives and analogs, *e.g.*, by recombinant means, are also provided.

The present invention also relates to therapeutic and diagnostic methods and compositions based on Delta proteins and nucleic acids. The invention provides for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: Delta proteins and analogs and derivatives (including fragments) thereof; antibodies thereto; nucleic acids encoding the Delta proteins, analogs, or derivatives; and Delta antisense nucleic acids. In a preferred embodiment, a Therapeutic of the invention is administered to treat a cancerous condition, or to prevent progression from a pre-neoplastic or non-malignant state into a neoplastic or a

malignant state. In other specific embodiments, a Therapeutic of the invention is administered to treat a nervous system disorder or to promote tissue regeneration and repair.

In one embodiment, Therapeutics which antagonize, or inhibit, Notch and/or Delta function (hereinafter "Antagonist Therapeutics") are administered for therapeutic effect. In another embodiment, Therapeutics which promote Notch and/or Delta function (hereinafter "Agonist Therapeutics") are administered for therapeutic effect.

Disorders of cell fate, in particular hyperproliferative (*e.g.*, cancer) or hypoproliferative disorders, involving aberrant or undesirable levels of expression or activity or localization of Notch and/or Delta protein can be diagnosed by detecting such levels, as described more fully *infra*.

In a preferred aspect, a Therapeutic of the invention is a protein consisting of at least a fragment (termed herein "adhesive fragment") of Delta which mediates binding to a Notch protein or a fragment thereof.

3.1. DEFINITIONS

As used herein, underscoring or italicizing the name of a gene shall indicate the gene, in contrast to its encoded protein product which is indicated by the name of the gene in the absence of any underscoring. For example, "*Delta*" shall mean the *Delta* gene, whereas "Delta" shall indicate the protein product of the *Delta* gene.

4. DESCRIPTION OF THE FIGURES

Figure 1A-1B. 1A. The DNA sequence of chick Delta (*C-Delta-1*) (SEQ ID NO:1). 1B. The DNA sequence of an alternatively spliced chick Delta (C-Delta-1) (SEQ ID NO:3).

Figure 2. The predicted amino acid sequence of chick Delta (C-Delta-1) (SEQ ID NO:2).

Figure 3. Predicted amino acid sequence of C-Delta-1 (SEQ ID NO:2), aligned with that of X-Delta-1 (*Xenopus* Delta;

SEQ ID NO:5) and *Drosophila* Delta (SEQ ID NO:6) and, indicating the conserved domain structures: EGF repeats, DSL domain, and transmembrane domain (TM). Conserved amino acids are boxed, and ● denote aligned and non-aligned N-terminal cysteine residues, respectively. Although the intracellular domains of C-Delta-1 and X-Delta-1 closely resemble each other, they show no significant homology to the corresponding part of *Drosophila* Delta.

Figure 4. Alignment of DSL domains from C-Delta-1 (SEQ ID NO:2), *Drosophila* Delta (SEQ ID NO:6) (Vässin et al., 1987, EMBO J. 6:3431-3440; Kopczynski et al., 1988, Genes Dev. 2:1723-1735), *Drosophila* Serrate (SEQ ID NO:7) (Fleming et al., 1990, Genes Dev. 4:2188-2201; Thomas et al., 1991, Development 111:749-761), C-Serrate-1 (SEQ ID NO:8) (Myat, Henrique, Ish-Horowicz and Lewis, in preparation), Apx-1 (SEQ ID NO:9) (Mello et al., 1994, Cell 77:95-106) and Lag-2 (SEQ ID NO:10) (Henderson et al., 1994, Development 120:2913-2924; Tax et al., 1994, Nature 368:150-154), showing the conserved Cysteine spacings, the amino acids that are conserved between presumed ligands for Notch-like proteins in *Drosophila* and vertebrates, and those that are further conserved in *C. elegans* ligands (boxes).

Figure 5A-5E. *C-Delta-1* and *C-Notch-1* expression correlate with onset of neurogenesis in the one-day (E1) neural plate. Anterior is to the left. Wholemount *in situ* hybridization specimens are shown in Figure 5*a-d*; 5*e* is a section. Figure 5*a*, At stage 7, *C-Notch-1* is expressed throughout most of the neural plate and part of the underlying presomitic mesoderm. Figure 5*b*, *C-Delta-1* at stage 7 is already detectable in the neural plate, in the future posterior hindbrain, just anterior to the first somite (white box). The posterior end of this neural domain is roughly level with the anterior margin of a domain of very strong expression in the underlying presomitic mesoderm (psm). Earlier expression in the neural plate may occur and be masked by expression in the underlying mesoderm (unpublished results). Figure 5*c*, Higher magnification view

of the area boxed in 5*b*, showing scattered cells in the neural plate expressing *C-Delta-1*. Figure 5*d*, At stage 8, *C-Delta-1* expression in the neural plate extends posteriorly as the neural plate develops. The domain of labelled neural plate cells visible in this photograph (bracketed) continues posteriorly over the presomitic mesoderm. Figure 5e, Parasagittal section of a stage 8 embryo showing that *C-Delta-1* is expressed in scattered cells of the neural plate (dorsal layer of tissue; bracketed), and broadly in the presomitic mesoderm (ventral layer). The plane of section is slightly oblique, missing the posterior part of the neural plate domain (cf. 5*d*).

Figure 6A-6C. *C-Delta-1*-expressing cells do not incorporate BrdU. Of 612 *C-Delta-1*' cells, 581 were $BrdU^-$ (76 sections; 6 embryos). Figure 6*a*, Diagram showing how phase in the cell cycle is related to apico-basal position of the nucleus for cells in the neuroepithelium; S-phase nuclei lie basally (Fujita, 1963, J. Comp. Neurol. 120:37-42; Biffo et al., 1992, Histochem. Cytochem. 40:535-540). Nuclei are indicated by shading. Figure 6*b*, Section through the neural tube of a stage 9 embryo labelled for 2 h with BrdU showing *C-Delta-1* expressing cells (dark on blue background) and BrdU-labelled nuclei (pink). Labelled nuclei are predominantly basal, where DNA synthesis occurs, yet basal *C-Delta-1*-expressing cells are unlabelled. Figure 6*c*, Section through a stage 9 embryo incubated for 4h: many labelled nuclei have exited S-phase and have moved towards the lumen, but *C-Delta-1*-expressing cells are still basal and not labelled with BrdU.

Figure 7. The DNA sequence of mouse *Delta* (M-*Delta*-1) (SEQ ID NO:11).

Figure 8. The predicted amino acid sequence of the mouse Delta (M-Delta-1) (SEQ ID NO:12).

Figure 9. An alignment of the predicted amino acid sequence of mouse M-Delta-1 (SEQ ID NO:12) with the chick C-Delta-1 (SEQ ID NO:2) which shows their extensive amino acid sequence identity. Identical amino acids are boxed. The

consensus sequence between the two genes is at the bottom (SEQ ID NO:13).

Figure 10. The DNA sequence of a PCR amplified fragment of human *Delta* (H-*Delta*-1) (SEQ ID NO:14) and the predicted amino acid sequences using the three available open reading frames, 2nd line (SEQ ID NO:15), 3rd line (SEQ ID NO:16), 4th line (SEQ ID NO:17).

Figure 11. An alignment of human H-Delta-1 (top line) and chick C-Delta-1 (bottom line). The predicted amino acid sequence of human Delta (SEQ ID NO:18) is shown in the top line. The sequence of human Delta was determined by "eye", in which the sequence of the appropriate reading frame was determined by maximizing homology with C-Delta-1. No single reading frame shown in Figure 10 gave the correct sequence due to errors in the DNA sequence of Figure 10 that caused reading frameshifts.

Figure 12A-12B. Figure 12A presents the contig DNA sequence of human *Delta* (H-*Delta*-1) (SEQ ID NO:33) from clone HDl 18. Figure 12B presents the nucleotide sequence shown in Figure 12A (top line, SEQ ID NO:33) and the deduced amino acid sequences using the three possible open reading frames, second line (SEQ ID NO:34), third line (SEQ ID NO:35), fourth line (SEQ ID NO:36). The amino acid sequence with the greatest homology to the mouse Delta-1 amino acid sequence is boxed. This boxed amino acid sequence is the predicted amino acid sequence of human Delta; where the reading frame shifts indicates where a sequencing error is present in the sequence. No single reading frame shown in Figure 12A gave an uninterrupted amino acid sequence due to errors in the DNA sequence that caused shifts in the reading frame. X indicates an undetermined amino acid; N indicates an undetermined nucleotide.

Figure 13. An alignment of mouse M-Delta-1 DNA sequence (top line, SEQ ID NO:37) and human H-Delta-1 DNA sequence (second line, SEQ ID NO:33) and their consensus sequence (third line, SEQ ID NO:38).

Figure 14. The composite human Delta (H-Delta-1) amino acid sequence (SEQ ID NOS:39-65, respectively) is presented, representing the boxed amino sequence from Figure 12B. ">" indicates that the sequence continues on the line below. "*" indicates a break in the sequence.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to nucleotide sequences of vertebrate *Delta* genes, and amino acid sequences of their encoded proteins. The invention further relates to fragments and other derivatives, and analogs, of vertebrate Delta proteins. Nucleic acids encoding such fragments or derivatives are also within the scope of the invention. The invention provides Delta genes and their encoded proteins of many different vertebrate species. The Delta genes of the invention include chick, mouse, and human *Delta* and related genes (homologs) in other vertebrate species. In specific embodiments, the Delta genes and proteins are from vertebrates, or more particularly, mammals. In a preferred embodiment of the invention, the Delta protein is a human protein. Production of the foregoing proteins and derivatives, *e.g.*, by recombinant methods, is provided.

The invention relates to Delta derivatives and analogs of the invention which are functionally active, i.e., they are capable of displaying one or more known functional activities associated with a full-length (wild-type) Delta protein. Such functional activities include but are not limited to antigenicity [ability to bind (or compete with Delta for binding) to an anti-Delta antibody], immunogenicity (ability to generate antibody which binds to Delta), ability to bind (or compete with Delta for binding) to Notch or other toporythmic proteins or fragments thereof ("adhesiveness"), ability to bind (or compete with Delta for binding) to a receptor for Delta, ability to affect cell fate differentiation, and therapeutic activity. "Toporythmic proteins" as used herein, refers to the protein products of *Notch*, *Delta*, *Serrate*, *Enhancer of split*, and *Deltex*, as well

as other members of this interacting gene family which may be identified, *e.g.*, by virtue of the ability of their gene sequences to hybridize, or their homology to Delta, Serrate, or Notch, or the ability of their genes to display phenotypic interactions.

The invention further relates to fragments (and derivatives and analogs thereof) of Delta which comprise one or more domains of the Delta protein, including but not limited to the intracellular domain, extracellular domain, DSL domain, region amino-terminal to the DSL domain, transmembrane domain, membrane-associated region, or one or more EGF-like (homologous) repeats of a Delta protein, or any combination of the foregoing.

Antibodies to vertebrate Delta, its derivatives and analogs, are additionally provided.

As demonstrated *infra*, Delta plays a critical role in development and other physiological processes, in particular, as a ligand to Notch, which is involved in cell fate (differentiation) determination. In particular, Delta is believed to play a major role in determining cell fates in the central nervous system. The nucleic acid and amino acid sequences and antibodies thereto of the invention can be used for the detection and quantitation of Delta mRNA and protein of human and other species, to study expression thereof, to produce Delta and fragments and other derivatives and analogs thereof, in the study and manipulation of differentiation and other physiological processes. The present invention also relates to therapeutic and diagnostic methods and compositions based on Delta proteins and nucleic acids. The invention provides for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: Delta proteins and analogs and derivatives (including fragments) thereof; antibodies thereto; nucleic acids encoding the Delta proteins, analogs, or derivatives; and Delta antisense nucleic acids. In a preferred embodiment, a Therapeutic of the invention is

administered to treat a cancerous condition, or to prevent progression from a pre-neoplastic or non-malignant state into a neoplastic or a malignant state. In other specific embodiments, a Therapeutic of the invention is administered to treat a nervous system disorder or to promote tissue regeneration and repair.

In one embodiment, Therapeutics which antagonize, or inhibit, Notch and/or Delta function (hereinafter "Antagonist Therapeutics") are administered for therapeutic effect. In another embodiment, Therapeutics which promote Notch and/or Delta function (hereinafter "Agonist Therapeutics") are administered for therapeutic effect.

Disorders of cell fate, in particular hyperproliferative (*e.g.*, cancer) or hypoproliferative disorders, involving aberrant or undesirable levels of expression or activity or localization of Notch and/or Delta protein can be diagnosed by detecting such levels, as described more fully *infra*.

In a preferred aspect, a Therapeutic of the invention is a protein consisting of at least a fragment (termed herein "adhesive fragment") of Delta which mediates binding to a Notch protein or a fragment thereof.

The invention is illustrated by way of examples *infra* which disclose, *inter alia*, the cloning of a chick *Delta* homolog (Section 6), the cloning of a mouse *Delta* homolog (Section 7), and the cloning of a human *Delta* homolog (Section 8).

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections which follow.

5.1. ISOLATION OF THE *DELTA* GENES

The invention relates to the nucleotide sequences of vertebrate *Delta* nucleic acids. In specific embodiments, human *Delta* nucleic acids comprise the cDNA sequences shown in Figure 10 (SEQ ID NO:14) or in Figure 12A (SEQ ID NO:33), or the coding regions thereof, or nucleic

acids encoding a vertebrate Delta protein (*e.g.*, having the sequence of SEQ ID NO:1, 3, 11, 14 or 33). The invention provides nucleic acids consisting of at least 8 nucleotides (*i.e.*, a hybridizable portion) of a vertebrate *Delta* sequence; in other embodiments, the nucleic acids consist of at least 25 (continuous) nucleotides, 50 nucleotides, 100 nucleotides, 150 nucleotides, or 200 nucleotides of a *Delta* sequence, or a full-length *Delta* coding sequence. The invention also relates to nucleic acids hybridizable to or complementary to the foregoing sequences or their complements. In specific aspects, nucleic acids are provided which comprise a sequence complementary to at least 10, 25, 50, 100, or 200 nucleotides or the entire coding region of a vertebrate *Delta* gene. In a specific embodiment, a nucleic acid which is hybridizable to a vertebrate (*e.g.*, mammalian) *Delta* nucleic acid (*e.g.*, having sequence SEQ ID NO:14 or SEQ ID NO:33, or an at least 10, 25, 50, 100, or 200 nucleotide portion thereof), or to a nucleic acid encoding a Delta derivative, under conditions of low stringency is provided. By way of example and not limitation, procedures using such conditions of low stringency are as follows (see also Shilo and Weinberg, 1981, Proc. Natl. Acad. Sci. USA 78:6789-6792): Filters containing DNA are pretreated for 6 h at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 μg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 μg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20 X 10^6 cpm ^{32}P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 h at 40°C, and then washed for 1.5 h at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 h at 60°C. Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film.

Other conditions of low stringency which may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations).

In another specific embodiment, a nucleic acid which is hybridizable to a vertebrate (*e.g.*, mammalian) Delta nucleic acid under conditions of high stringency is provided. By way of example and not limitation, procedures using such conditions of high stringency are as follows: Prehybridization of filters containing DNA is carried out for 8 h to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65°c in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}p-labeled probe. Washing of filters is done at 37°C for 1 h in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 min before autoradiography. Other conditions of high stringency which may be used are well known in the art.

Nucleic acids encoding fragments and derivatives of vertebrate Delta proteins (see Section 5.6), and *Delta* antisense nucleic acids (see Section 5.11) are additionally provided. As is readily apparent, as used herein, a "nucleic acid encoding a fragment or portion of a Delta protein" shall be construed as referring to a nucleic acid encoding only the recited fragment or portion of the Delta protein and not the other contiguous portions of the Delta protein as a continuous sequence.

Fragments of vertebrate *Delta* nucleic acids comprising regions of homology to other toporythmic proteins are also provided. The DSL regions (regions of homology with *Drosophila* Serrate and Delta) of Delta proteins of other species are also provided. Nucleic acids encoding conserved regions between Delta and Serrate, such as those shown in Figures 3 and 8 are also provided.

Specific embodiments for the cloning of a vertebrate *Delta* gene, presented as a particular example but not by way of limitation, follows:

For expression cloning (a technique commonly known in the art), an expression library is constructed by methods known in the art. For example, mRNA (*e.g.*, human) is isolated, cDNA is made and ligated into an expression vector (*e.g.*, a bacteriophage derivative) such that it is capable of being expressed by the host cell into which it is then introduced. Various screening assays can then be used to select for the expressed Delta product. In one embodiment, anti-Delta antibodies can be used for selection.

In another preferred aspect, PCR is used to amplify the desired sequence in a genomic or cDNA library, prior to selection. Oligonucleotide primers representing known Delta sequences (preferably vertebrate sequences) can be used as primers in PCR. In a preferred aspect, the oligonucleotide primers represent at least part of the Delta conserved segments of strong homology between *Serrate* and *Delta*. The synthetic oligonucleotides may be utilized as primers to amplify by PCR sequences from a source (RNA or DNA), preferably a cDNA library, of potential interest. PCR can be carried out, *e.g.*, by use of a Perkin-Elmer Cetus thermal cycler and Taq polymerase (Gene Amp™). The DNA being amplified can include mRNA or cDNA or genomic DNA from any eukaryotic species. One can choose to synthesize several different degenerate primers, for use in the PCR reactions. It is also possible to vary the stringency of hybridization conditions used in priming the PCR reactions, to allow for greater or lesser degrees of nucleotide sequence similarity between the known *Delta* nucleotide sequence and the nucleic acid homolog being isolated. For cross species hybridization, low stringency conditions are preferred. For same species hybridization, moderately stringent conditions are preferred. After successful amplification of a segment of a *Delta* homolog, that segment may be molecularly cloned and sequenced, and utilized as a probe to isolate a complete

cDNA or genomic clone. This, in turn, will permit the determination of the gene's complete nucleotide sequence, the analysis of its expression, and the production of its protein product for functional analysis, as described *infra*. In this fashion, additional genes encoding Delta proteins may be identified. Such a procedure is presented by way of example in various examples sections *infra*.

The above-methods are not meant to limit the following general description of methods by which clones of *Delta* may be obtained.

Any vertebrate cell potentially can serve as the nucleic acid source for the molecular cloning of the *Delta* gene. The nucleic acid sequences encoding Delta can be isolated from mammalian, human, porcine, bovine, feline, avian, equine, canine, as well as additional primate sources, etc. For example, we have amplified fragments of the Delta gene in mouse, chicken, and human, by PCR using cDNA libraries with *Delta* primers. The DNA may be obtained by standard procedures known in the art from cloned DNA (*e.g.*, a DNA "library"), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired cell. (See, for example, Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K. Vol. I, II.) Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences. Whatever the source, the gene should be molecularly cloned into a suitable vector for propagation of the gene.

In the molecular cloning of the gene from genomic DNA, DNA fragments are generated, some of which will encode the desired gene. The DNA may be cleaved at specific sites using various restriction enzymes. Alternatively, one may use DNAse in the presence of manganese to fragment the DNA, or the DNA can be physically sheared, as for example, by

sonication. The linear DNA fragments can then be separated according to size by standard techniques, including but not limited to, agarose and polyacrylamide gel electrophoresis and column chromatography.

Once the DNA fragments are generated, identification of the specific DNA fragment containing the desired gene may be accomplished in a number of ways. For example, if an amount of a portion of a *Delta* (of any species) gene or its specific RNA, or a fragment thereof, e.g., an extracellular domain (see Section 5.6), is available and can be purified and labeled, the generated DNA fragments may be screened by nucleic acid hybridization to the labeled probe (Benton, W. and Davis, R., 1977, Science 196:180; Grunstein, M. And Hogness, D., 1975, Proc. Natl. Acad. Sci. U.S.A. 72:3961). Those DNA fragments with substantial homology to the probe will hybridize. It is also possible to identify the appropriate fragment by restriction enzyme digestion(s) and comparison of fragment sizes with those expected according to a known restriction map if such is available. Further selection can be carried out on the basis of the properties of the gene. Alternatively, the presence of the gene may be detected by assays based on the physical, chemical, or immunological properties of its expressed product. For example, cDNA clones, or DNA clones which hybrid-select the proper mRNAs, can be selected which produce a protein that, e.g., has similar or identical electrophoretic migration, isolectric focusing behavior, proteolytic digestion maps, binding activity, *in vitro* aggregation activity ("adhesiveness") or antigenic properties as known for Delta. If an antibody to Delta is available, the Delta protein may be identified by binding of labeled antibody to the putatively Delta synthesizing clones, in an ELISA (enzyme-linked immunosorbent assay)-type procedure.

The *Delta* gene can also be identified by mRNA selection by nucleic acid hybridization followed by *in vitro* translation. In this procedure, fragments are used to isolate complementary mRNAs by hybridization. Such DNA

fragments may represent available, purified Delta DNA of another species (e.g., Drosophila). Immunoprecipitation analysis or functional assays (e.g., aggregation ability in vitro; binding to receptor; see infra) of the in vitro translation products of the isolated products of the isolated mRNAs identifies the mRNA and, therefore, the complementary DNA fragments that contain the desired sequences. In addition, specific mRNAs may be selected by adsorption of polysomes isolated from cells to immobilized antibodies specifically directed against Delta protein. A radiolabelled Delta cDNA can be synthesized using the selected mRNA (from the adsorbed polysomes) as a template. The radiolabelled mRNA or cDNA may then be used as a probe to identify the Delta DNA fragments from among other genomic DNA fragments.

Alternatives to isolating the Delta genomic DNA include, but are not limited to, chemically synthesizing the gene sequence itself from a known sequence or making cDNA to the mRNA which encodes the Delta protein. For example, RNA for cDNA cloning of the Delta gene can be isolated from cells which express Delta. Other methods are possible and within the scope of the invention.

The identified and isolated gene can then be inserted into an appropriate cloning vector. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as PBR322 or pUC plasmid derivatives. The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may

comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and *Delta* gene may be modified by homopolymeric tailing. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

In an alternative method, the desired gene may be identified and isolated after insertion into a suitable cloning vector in a "shot gun" approach. Enrichment for the desired gene, for example, by size fractionation, can be done before insertion into the cloning vector.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated *Delta* gene, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

The Delta sequences provided by the instant invention include those nucleotide sequences encoding substantially the same amino acid sequences as found in native vertebrate Delta proteins, and those encoded amino acid sequences with functionally equivalent amino acids, all as described in Section 5.6 *infra* for Delta derivatives.

5.2. EXPRESSION OF THE *DELTA* GENES

The nucleotide sequence coding for a vertebrate Delta protein or a functionally active fragment or other derivative thereof (see Section 5.6), can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The necessary transcriptional and translational signals can also be supplied by the native *Delta* gene and/or its flanking regions. A variety of host-vector systems may be utilized to

express the protein-coding sequence. These include but are not limited to mammalian cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (*e.g.*, baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used. In a specific embodiment, the adhesive portion of the *Delta* gene is expressed. In other specific embodiments, the human *Delta* gene is expressed, or a sequence encoding a functionally active portion of human Delta. In yet another embodiment, a fragment of Delta comprising the extracellular domain, or other derivative, or analog of Delta is expressed.

Any of the methods previously described for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a chimeric gene consisting of appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequence encoding a Delta protein or peptide fragment may be regulated by a second nucleic acid sequence so that the Delta protein or peptide is expressed in a host transformed with the recombinant DNA molecule. For example, expression of a Delta protein may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control *Delta* gene expression include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3′ long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982,

Nature 296:39-42); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the *tac* promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25); see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; plant expression vectors comprising the nopaline synthetase promoter region (Herrera-Estrella et al., Nature 303:209-213) or the cauliflower mosaic virus 35S RNA promoter (Gardner, et al., 1981, Nucl. Acids Res. 9:2871), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella et al., 1984, Nature 310:115-120); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58; alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells

(Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94; myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

Expression vectors containing *Delta* gene inserts can be identified by three general approaches: (a) nucleic acid hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a foreign gene inserted in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted toporythmic gene. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (*e.g.*, thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign genes in the vector. For example, if the *Delta* gene is inserted within the marker gene sequence of the vector, recombinants containing the *Delta* insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the foreign gene product expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the *Delta* gene product *in vitro* assay systems, *e.g.*, aggregation (binding) with Notch, binding to a receptor, binding with antibody.

Once a particular recombinant DNA molecule is identified and isolated, several methods known in the art may be used to propagate it. Once a suitable host system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As

previously explained, the expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (*e.g.*, lambda), and plasmid and cosmid DNA vectors, to name but a few.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered Delta protein may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, cleavage [*e.g.*, of signal sequence]) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce an unglycosylated core protein product. Expression in yeast will produce a glycosylated product. Expression in mammalian cells can be used to ensure "native" glycosylation of a heterologous mammalian Delta protein. Furthermore, different vector/host expression systems may effect processing reactions such as proteolytic cleavages to different extents.

In other specific embodiments, the Delta protein, fragment, analog, or derivative may be expressed as a fusion, or chimeric protein product (comprising the protein, fragment, analog, or derivative joined via a peptide bond to a heterologous protein sequence (of a different protein)). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a

chimeric product may be made by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer.

Both cDNA and genomic sequences can be cloned and expressed.

5

5.3. IDENTIFICATION AND PURIFICATION OF THE *DELTA* GENE PRODUCTS

In particular aspects, the invention provides amino acid sequences of a vertebrate Delta, preferably a human Delta, and fragments and derivatives thereof which comprise an antigenic determinant (*i.e.*, can be recognized by an antibody) or which are otherwise functionally active, as well as nucleic acid sequences encoding the foregoing. "Functionally active" material as used herein refers to that material displaying one or more known functional activities associated with a full-length (wild-type) Delta protein, *e.g.*, binding to Notch or a portion thereof, binding to any other Delta ligand, antigenicity (binding to an anti-Delta antibody), etc.

In specific embodiments, the invention provides fragments of a Delta protein consisting of at least 6 amino acids, 10 amino acids, 25 amino acids, 50 amino acids, or of at least 75 amino acids. Molecules comprising such fragments are also provided. In other embodiments, the proteins comprise or consist essentially of an extracellular domain, DSL domain, epidermal growth factor-like repeat (ELR) domain, one or any combination of ELRs, transmembrane domain, or intracellular (cytoplasmic) domain, or a portion which binds to Notch, or any combination of the foregoing, of a vertebrate Delta protein. Fragments, or proteins comprising fragments, lacking some or all of the foregoing regions of a Delta protein are also provided. Nucleic acids encoding the foregoing are provided.

Once a recombinant which expresses the *Delta* gene sequence is identified, the gene product can be analyzed. This is achieved by assays based on the physical or functional properties of the product, including radioactive

labelling of the product followed by analysis by gel electrophoresis, immunoassay, etc.

Once the Delta protein is identified, it may be isolated and purified by standard methods including chromatography (*e.g.*, ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. The functional properties may be evaluated using any suitable assay (see Section 5.7).

Alternatively, once a Delta protein produced by a recombinant is identified, the amino acid sequence of the protein can be deduced from the nucleotide sequence of the chimeric gene contained in the recombinant. As a result, the protein can be synthesized by standard chemical methods known in the art (*e.g.*, see Hunkapiller, M., et al., 1984, Nature 310:105-111).

In a specific embodiment of the present invention, such Delta proteins, whether produced by recombinant DNA techniques or by chemical synthetic methods, include but are not limited to those containing, as a primary amino acid sequence, all or part of the amino acid sequences substantially as depicted in Figures 2, 8, 11 or 14 (SEQ ID NOS:2, 10, 16 and 39-65), as well as fragments and other derivatives, and analogs thereof.

5.4. STRUCTURE OF THE *DELTA* GENES AND PROTEINS

The structure of the vertebrate *Delta* genes and proteins can be analyzed by various methods known in the art.

5.4.1. GENETIC ANALYSIS

The cloned DNA or cDNA corresponding to the *Delta* gene can be analyzed by methods including but not limited to Southern hybridization (Southern, E.M., 1975, J. Mol. Biol. 98:503-517), Northern hybridization (see *e.g.*, Freeman et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:4094-4098), restriction endonuclease mapping (Maniatis, T., 1982, Molecular Cloning, A Laboratory, Cold Spring Harbor, New

York), and DNA sequence analysis. Polymerase chain reaction (PCR; U.S. Patent Nos. 4,683,202, 4,683,195 and 4,889,818; Gyllenstein et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7652-7656; Ochman et al., 1988, Genetics 120:621-623; Loh et al., 1989, Science 243:217-220) followed by Southern hybridization with a *Delta*-specific probe can allow the detection of the *Delta* gene in DNA from various cell types. Methods of amplification other than PCR are commonly known and can also be employed. In one embodiment, Southern hybridization can be used to determine the genetic linkage of *Delta*. Northern hybridization analysis can be used to determine the expression of the *Delta* gene. Various cell types, at various states of development or activity can be tested for *Delta* expression. Examples of such techniques and their results are described in Section 6, *infra*. The stringency of the hybridization conditions for both Southern and Northern hybridization can be manipulated to ensure detection of nucleic acids with the desired degree of relatedness to the specific *Delta* probe used.

Restriction endonuclease mapping can be used to roughly determine the genetic structure of the *Delta* gene. Restriction maps derived by restriction endonuclease cleavage can be confirmed by DNA sequence analysis.

DNA sequence analysis can be performed by any techniques known in the art, including but not limited to the method of Maxam and Gilbert (1980, Meth. Enzymol. 65:499-560), the Sanger dideoxy method (Sanger, F., et al., 1977, Proc. Natl. Acad. Sci. U.S.A. 74:5463), the use of T7 DNA polymerase (Tabor and Richardson, U.S. Patent No. 4,795,699), or use of an automated DNA sequenator (e.g., Applied Biosystems, Foster City, CA).

5.4.2. PROTEIN ANALYSIS

The amino acid sequence of the Delta protein can be derived by deduction from the DNA sequence, or alternatively, by direct sequencing of the protein, e.g., with an automated amino acid sequencer. The amino acid sequence of a

representative Delta protein comprises the sequence substantially as depicted in Figure 2, and detailed in Section 6, *infra*, with the representative mature protein that shown by amino acid numbers 1-728.

The Delta protein sequence can be further characterized by a hydrophilicity analysis (Hopp, T. and Woods, K., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:3824). A hydrophilicity profile can be used to identify the hydrophobic and hydrophilic regions of the Delta protein and the corresponding regions of the gene sequence which encode such regions. Hydrophilic regions are more likely to be immunogenic.

Secondary structural analysis (Chou, P. and Fasman, G., 1974, Biochemistry 13:222) can also be done, to identify regions of Delta that assume specific secondary structures.

Manipulation, translation, and secondary structure prediction, as well as open reading frame prediction and plotting, can also be accomplished using computer software programs available in the art.

Other methods of structural analysis can also be employed. These include but are not limited to X-ray crystallography (Engstom, A., 1974, Biochem. Exp. Biol. 11:7-13) and computer modeling (Fletterick, R. and Zoller, M. (eds.), 1986, Computer Graphics and Molecular Modeling, *in* Current Communications in Molecular Biology, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York).

5.5. GENERATION OF ANTIBODIES TO DELTA PROTEINS AND DERIVATIVES THEREOF

According to the invention, a vertebrate Delta protein, its fragments or other derivatives, or analogs thereof, may be used as an immunogen to generate antibodies which recognize such an immunogen. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library. In a specific embodiment, antibodies to human Delta are

produced. In another embodiment, antibodies to the extracellular domain of Delta are produced. In another embodiment, antibodies to the intracellular domain of Delta are produced.

Various procedures known in the art may be used for the production of polyclonal antibodies to a Delta protein or derivative or analog. In a particular embodiment, rabbit polyclonal antibodies to an epitope of the Delta protein encoded by a sequence depicted in Figures 1a, 1b, 7 or 11, or a subsequence thereof, can be obtained. For the production of antibody, various host animals can be immunized by injection with the native Delta protein, or a synthetic version, or derivative (*e.g.*, fragment) thereof, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

For preparation of monoclonal antibodies directed toward a Delta protein sequence or analog thereof, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing recent technology (PCT/US90/02545). According to the invention, human antibodies may be used and can be obtained by using human

hybridomas (Cote et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:2026-2030) or by transforming human B cells with EBV virus *in vitro* (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, pp. 77-96). In fact, according to the invention, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851-6855; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing the genes from a mouse antibody molecule specific for Delta together with genes from a human antibody molecule of appropriate biological activity can be used; such antibodies are within the scope of this invention.

According to the invention, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce Delta-specific single chain antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries (Huse et al., 1989, Science 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for Delta proteins, derivatives, or analogs.

Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, *e.g.* ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies which recognize a specific domain of a vertebrate Delta protein, one may assay generated hybridomas for a product which binds to a Delta fragment containing such domain. For selection of an antibody

immunospecific to human Delta, one can select on the basis of positive binding to human Delta and a lack of binding to *Drosophila* Delta.

The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the protein sequences of the invention (*e.g.*, see Section 5.7, *infra*), *e.g.*, for imaging these proteins, measuring levels thereof in appropriate physiological samples, in diagnostic methods, etc.

Antibodies specific to a domain of a Delta protein are also provided. In a specific embodiment, antibodies which bind to a Notch-binding fragment of Delta are provided.

In another embodiment of the invention (see *infra*), anti-Delta antibodies and fragments thereof containing the binding domain are Therapeutics.

5.6. DELTA PROTEINS, DERIVATIVES AND ANALOGS

The invention further relates to vertebrate (*e.g.*, mammalian) Delta proteins, and derivatives (including but not limited to fragments) and analogs of vertebrate Delta proteins. Nucleic acids encoding Delta protein derivatives and protein analogs are also provided. In one embodiment, the Delta proteins are encoded by the Delta nucleic acids described in Section 5.1 *supra*. In particular aspects, the proteins, derivatives, or analogs are of mouse, chicken, rat, pig, cow, dog, monkey, or human Delta proteins. In a specific embodiment, a mature, full-length vertebrate Delta protein is provided. In one embodiment, a vertebrate Delta protein lacking only the signal sequence (approximately the first 17 amino-terminal amino acids) is provided.

The production and use of derivatives and analogs related to *Delta* are within the scope of the present invention. In a specific embodiment, the derivative or analog is functionally active, i.e., capable of exhibiting one or more functional activities associated with a full-length, wild-type Delta protein. As one example, such derivatives or analogs which have the desired immunogenicity

or antigenicity can be used, for example, in immunoassays, for immunization, for inhibition of Delta activity, etc. Such molecules which retain, or alternatively inhibit, a desired Delta property, *e.g.*, binding to Notch or other toporythmic proteins, binding to a cell-surface receptor, can be used as inducers, or inhibitors, respectively, of such property and its physiological correlates. A specific embodiment relates to a Delta fragment that can be bound by an anti-Delta antibody but cannot bind to a Notch protein or other toporythmic protein. Derivatives or analogs of Delta can be tested for the desired activity by procedures known in the art, including but not limited to the assays described in Section 5.7.

In particular, Delta derivatives can be made by altering *Delta* sequences by substitutions, additions or deletions that provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as a *Delta* gene may be used in the practice of the present invention. These include but are not limited to nuclectide sequences comprising all or portions of *Delta* genes which are altered by the substitution of different ccdons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change. Likewise, the Delta derivatives of the invention include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of a Delta protein including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a silent change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine,

isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

In a specific embodiment of the invention, proteins consisting of or comprising a fragment of a vertebrate Delta protein consisting of at least 10 (continuous) amino acids of the Delta protein is provided. In other embodiments, the fragment consists of at least 20 or 50 amino acids of the Delta protein. In specific embodiments, such fragments are not larger than 35, 100 or 200 amino acids. Derivatives or analogs of Delta include but are not limited to those peptides which are substantially homologous to a vertebrate Delta protein or fragments thereof (*e.g.*, at least 30%, 50%, 70%, or 90% identity over an amino acid sequence of identical size -- *e.g.*, comprising a domain) or whose encoding nucleic acid is capable of hybridizing to a coding *Delta* sequence.

The Delta derivatives and analogs of the invention can be produced by various methods known in the art. The manipulations which result in their production can occur at the gene or protein level. For example, the cloned *Delta* gene sequence can be modified by any of numerous strategies known in the art (Maniatis, T., 1990, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The sequence can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated *in vitro*. In the production of the gene encoding a derivative or analog of Delta, care should be taken to ensure that the modified gene remains within the same translational reading frame as Delta, uninterrupted by translational stop signals, in the gene region where the desired Delta activity is encoded.

Additionally, the Delta-encoding nucleic acid sequence can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy preexisting ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art can be used, including but not limited to, *in vitro* site-directed mutagenesis (Hutchinson, C., et al., 1978, J. Biol. Chem 253:6551), use of TAB® linkers (Pharmacia), etc. PCR primers containing sequence changes can be used in PCR to introduce such changes into the amplified fragments.

Manipulations of the Delta sequence may also be made at the protein level. Included within the scope of the invention are Delta protein fragments or other derivatives or analogs which are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

In addition, analogs and derivatives of Delta can be chemically synthesized. For example, a peptide corresponding to a portion of a Delta protein which comprises the desired domain (see Section 5.6.1), or which mediates the desired aggregation activity *in vitro*, or binding to a receptor, can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the Delta sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, α-amino isobutyric acid, 4-aminobutyric acid,

hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, and Nα-methyl amino acids.

In a specific embodiment, the Delta derivative is a chimeric, or fusion, protein comprising a vertebrate Delta protein or fragment thereof (preferably consisting of at least a domain or motif of the Delta protein, or at least 10 amino acids of the Delta protein) joined at its amino- or carboxy-terminus via a peptide bond to an amino acid sequence of a different protein. In one embodiment, such a chimeric protein is produced by recombinant expression of a nucleic acid encoding the protein (comprising a Delta-coding sequence joined in-frame to a coding sequence for a different protein). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer. In a specific embodiment, a chimeric nucleic acid encoding a mature Delta protein with a heterologous signal sequence is expressed such that the chimeric protein is expressed and processed by the cell to the mature Delta protein. As another example, and not by way of limitation, a recombinant molecule can be constructed according to the invention, comprising coding portions of both *Delta* and another toporythmic gene, *e.g.*, Serrate. The encoded protein of such a recombinant molecule could exhibit properties associated with both Serrate and Delta and portray a novel profile of biological activities, including agonists as well as antagonists. The primary sequence of Delta and Serrate may also be used to predict tertiary structure of the molecules using computer simulation (Hopp and Woods, 1981, Proc. Natl. Acad. Sci. U.S.A. 78:3824-3828); *Delta/Serrate* chimeric

recombinant genes could be designed in light of correlations between tertiary structure and biological function. Likewise, chimeric genes comprising portions of *Delta* fused to any heterologous protein-encoding sequences may be constructed. A specific embodiment relates to a chimeric protein comprising a fragment of Delta of at least six amino acids.

In another specific embodiment, the Delta derivative is a fragment of vertebrate Delta comprising a region of homology with another toporythmic protein. As used herein, a region of a first protein shall be considered "homologous" to a second protein when the amino acid sequence of the region is at least 30% identical or at least 75% either identical or involving conservative changes, when compared to any sequence in the second protein of an equal number of amino acids as the number contained in the region. For example, such a Delta fragment can comprise one or more regions homologous to Serrate, including but not limited to the DSL domain or a portion thereof.

Other specific embodiments of derivatives and analogs are described in the subsections below and examples sections *infra*.

5.6.1. DERIVATIVES OF DELTA CONTAINING ONE OR MORE DOMAINS OF THE PROTEIN

In a specific embodiment, the invention relates to vertebrate Delta derivatives and analogs, in particular Delta fragments and derivatives of such fragments, that comprise, or alternatively consist of, one or more domains of the Delta protein, including but not limited to the extracellular domain, signal sequence, region amino-terminal to the DSL domain, DSL domain, ELR domain, transmembrane domain, intracellular domain, and one or more of the EGF-like repeats (ELR) of the Delta protein (*e.g.*, ELRs 1-9), or any combination of the foregoing. In particular examples relating to the chick and mouse Delta proteins, such domains are identified in Examples Section 6 and 7, respectively, and

in Figures 3 and 9. Thus, by way of example is provided, a molecule comprising an extracellular domain (approximately amino acids 1-545), signal sequence (approximately amino acids 1-17), region amino-terminal to the DSL domain (approximately amino acids 1-178), the DSL domain (approximately amino acids 179-223), EGF1 (approximately amino acids 229-260), EGF2 (approximately amino acids 261-292), EGF3 (approximately amino acids 293-332), EGF4 (approximately amino acids 333-370), EGF5 (approximately amino acids 371-409), EGF6 (approximately amino acids 410-447), EGF7 (approximately amino acids 448-485), EGF8 (approximately amino acids 486-523), transmembrane domain, and intracellular (cytoplasmic) domain (approximately amino acids 555-728) of a vertebrate Delta.

In a specific embodiment, the molecules comprising specific fragments of vertebrate Delta are those comprising fragments in the respective Delta protein most homologous to specific fragments of the *Drosophila* or chick Delta protein. In particular embodiments, such a molecule comprises or consists of the amino acid sequences of SEQ ID NO:2 or 16. Alternatively, a fragment comprising a domain of a Delta homolog can be identified by protein analysis methods as described in Section 5.3.2.

5.6.2. DERIVATIVES OF DELTA THAT MEDIATE BINDING TO TOPORYTHMIC PROTEIN DOMAINS

The invention also provides for vertebrate Delta fragments, and analogs or derivatives of such fragments, which mediate binding to toporythmic proteins (and thus are termed herein "adhesive"), and nucleic acid sequences encoding the foregoing.

In a particular embodiment, the adhesive fragment of a Delta protein comprises the DSL domain, or a portion thereof. Subfragments within the DSL domain that mediate binding to Notch can be identified by analysis of constructs expressing deletion mutants.

The ability to bind to a toporythmic protein (preferably Notch) can be demonstrated by *in vitro* aggregation assays with cells expressing such a toporythmic protein as well as cells expressing Delta or a Delta derivative (See Section 5.7). That is, the ability of a Delta fragment to bind to a Notch protein can be demonstrated by detecting the ability of the Delta fragment, when expressed on the surface of a first cell, to bind to a Notch protein expressed on the surface of a second cell.

The nucleic acid sequences encoding toporythmic proteins or adhesive domains thereof, for use in such assays, can be isolated from human, porcine, bovine, feline, avian, equine, canine, or insect, as well as primate sources and any other species in which homologs of known toporythmic genes can be identified.

5.7. ASSAYS OF DELTA PROTEINS, DERIVATIVES AND ANALOGS

The functional activity of vertebrate Delta proteins, derivatives and analogs can be assayed by various methods.

For example, in one embodiment, where one is assaying for the ability to bind or compete with wild-type Delta for binding to anti-Delta antibody, various immunoassays known in the art can be used, including but not limited to competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the

primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labelled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where one is assaying for the ability to mediate binding to a toporythmic protein, *e.g.*, Notch, one can carry out an *in vitro* aggregation assay (see Fehon et al., 1990, Cell 61:523-534; Rebay et al., 1991, Cell 67:687-699).

In another embodiment, where a receptor for Delta is identified, receptor binding can be assayed, *e.g.*, by means well-known in the art. In another embodiment, physiological correlates of Delta binding to cells expressing a Delta receptor (signal transduction) can be assayed.

In another embodiment, in insect or other model systems, genetic studies can be done to study the phenotypic effect of a Delta mutant that is a derivative or analog of wild-type *Delta*.

Other methods will be known to the skilled artisan and are within the scope of the invention.

5.8. THERAPEUTIC USES

The invention provides for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: Delta proteins and analogs and derivatives (including fragments) thereof (*e.g.*, as described hereinabove); antibodies thereto (as described hereinabove); nucleic acids encoding the Delta proteins, analogs, or derivatives (*e.g.*, as described hereinabove); and *Delta* antisense nucleic acids. As stated *supra*, the Antagonist Therapeutics of the invention are those Therapeutics which antagonize, or inhibit, a Delta function and/or Notch function (since Delta is a Notch ligand). Such Antagonist Therapeutics are most preferably identified by use

of known convenient *in vitro* assays, *e.g.*, based on their ability to inhibit binding of Delta to another protein (*e.g.*, a Notch protein), or inhibit any known Notch or Delta function as preferably assayed *in vitro* or in cell culture, although genetic assays (*e.g.*, in *Drosophila*) may also be employed. In a preferred embodiment, the Antagonist Therapeutic is a protein or derivative thereof comprising a functionally active fragment such as a fragment of Delta which mediates binding to Notch, or an antibody thereto. In other specific embodiments, such an Antagonist Therapeutic is a nucleic acid capable of expressing a molecule comprising a fragment of Delta which binds to Notch, or a *Delta* antisense nucleic acid (see Section 5.11 herein). It should be noted that preferably, suitable *in vitro* or *in vivo* assays, as described *infra*, should be utilized to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue, since the developmental history of the tissue may determine whether an Antagonist or Agonist Therapeutic is desired.

In addition, the mode of administration, *e.g.*, whether administered in soluble form or administered via its encoding nucleic acid for intracellular recombinant expression, of the Delta protein or derivative can affect whether it acts as an agonist or antagonist.

In another embodiment of the invention, a nucleic acid containing a portion of a *Delta* gene is used, as an Antagonist Therapeutic, to promote *Delta* inactivation by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438).

The Agonist Therapeutics of the invention, as described *supra*, promote Delta function. Such Agonist Therapeutics include but are not limited to proteins and derivatives comprising the portions of Notch that mediate binding to Delta, and nucleic acids encoding the foregoing (which can be administered to express their encoded products *in vivo*).

Further descriptions and sources of Therapeutics of the inventions are found in Sections 5.1 through 5.7 herein.

Molecules which retain, or alternatively inhibit, a desired Delta property, *e.g.*, binding to Notch, binding to an intracellular ligand, can be used therapeutically as inducers, or inhibitors, respectively, of such property and its physiological correlates. In a specific embodiment, a peptide (*e.g.*, in the range of 6-50 or 15-25 amino acids; and particularly of about 10, 15, 20 or 25 amino acids) containing the sequence of a portion of Delta which binds to Notch is used to antagonize Notch function. In a specific embodiment, such an Antagonist Therapeutic is used to treat or prevent human or other malignancies associated with increased Notch expression (*e.g.*, cervical cancer, colon cancer, breast cancer, squamous adenocarcimas (see *infra*)). Derivatives or analogs of Delta can be tested for the desired activity by procedures known in the art, including but not limited to the assays described in the examples *infra*. For example, molecules comprising Delta fragments which bind to Notch EGF-repeats (ELR) 11 and 12 and which are smaller than a DSL domain, can be obtained and selected by expressing deletion mutants and assaying for binding of the expressed product to Notch by any of the several methods (*e.g.*, *in vitro* cell aggregation assays, interaction trap system), some of which are described in the Examples Sections *infra*. In one specific embodiment, peptide libraries can be screened to select a peptide with the desired activity; such screening can be carried out by assaying, *e.g.*, for binding to Notch or a molecule containing the Notch ELR 11 and 12 repeats.

Other Therapeutics include molecules that bind to a vertebrate Delta protein. Thus, the invention also provides a method for identifying such molecules. Such molecules can be identified by a method comprising contacting a plurality of molecules (*e.g.*, in a peptide library, or combinatorial chemical library) with the Delta protein under conditions conducive to binding, and recovering any molecules that bind to the Delta protein.

The Agonist and Antagonist Therapeutics of the invention have therapeutic utility for disorders of cell fate. The Agonist Therapeutics are administered therapeutically (including prophylactically): (1) in diseases or disorders involving an absence or decreased (relative to normal, or desired) levels of Notch or Delta function, for example, in patients where Notch or Delta protein is lacking, genetically defective, biologically inactive or underactive, or underexpressed; and (2) in diseases or disorders wherein of Delta agonist administration. The absence or decreased levels in Notch or Delta function can be readily detected, *e.g.*, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for protein levels, structure and/or activity of the expressed Notch or Delta protein. Many methods standard in the art can be thus employed, including but not limited to immunoassays to detect and/or visualize Notch or Delta protein (*e.g.*, Western blot, immunoprecipitation followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect Notch or Delta expression by detecting and/or visualizing respectively Notch or Delta mRNA (*e.g.*, Northern assays, dot blots, *in situ* hybridization, etc.)

In vitro assays which can be used to determine whether administration of a specific Agonist Therapeutic or Antagonist Therapeutic is indicated, include *in vitro* cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a Therapeutic, and the effect of such Therapeutic upon the tissue sample is observed. In one embodiment, where the patient has a malignancy, a sample of cells from such malignancy is plated out or grown in culture, and the cells are then exposed to a Therapeutic. A Therapeutic which inhibits survival or growth of the malignant cells (*e.g.*, by promoting terminal differentiation) is selected for therapeutic use *in vivo*. Many assays standard in the art can

be used to assess such survival and/or growth; for example, cell proliferation can be assayed by measuring ^{3}H-thymidine incorporation, by direct cell count, by detecting changes in transcriptional activity of known genes such as proto-oncogenes (e.g., *fos, myc*) or cell cycle markers; cell viability can be assessed by trypan blue staining, differentiation can be assessed visually based on changes in morphology, etc. In a specific aspect, the malignant cell cultures are separately exposed to (1) an Agonist Therapeutic, and (2) an Antagonist Therapeutic; the result of the assay can indicate which type of Therapeutic has therapeutic efficacy.

In another embodiment, a Therapeutic is indicated for use which exhibits the desired effect, inhibition or promotion of cell growth, upon a patient cell sample from tissue having or suspected of having a hyper- or hypoproliferative disorder, respectively. Such hyper- or hypoproliferative disorders include but are not limited to those described in Sections 5.8.1 through 5.8.3 *infra*.

In another specific embodiment, a Therapeutic is indicated for use in treating nerve injury or a nervous system degenerative disorder (see Section 5.8.2) which exhibits *in vitro* promotion of nerve regeneration/neurite extension from nerve cells of the affected patient type.

In addition, administration of an Antagonist Therapeutic of the invention is also indicated in diseases or disorders determined or known to involve a Notch or Delta dominant activated phenotype ("gain of function" mutations.) Administration of an Agonist Therapeutic is indicated in diseases or disorders determined or known to involve a Notch or Delta dominant negative phenotype ("loss of function" mutations). The functions of various structural domains of the Notch protein have been investigated *in vivo*, by ectopically expressing a series of *Drosophila Notch* deletion mutants under the hsp70 heat-shock promoter, as well as eye-specific promoters (see Rebay et al., 1993, Cell 74:319-329). Two classes of dominant phenotypes were observed, one

suggestive of *Notch* loss-of function mutations and the other of *Notch* gain-of-function mutations. Dominant "activated" phenotypes resulted from overexpression of a protein lacking most extracellular sequences, while dominant "negative" phenotypes resulted from overexpression of a protein lacking most intracellular sequences. The results indicated that Notch functions as a receptor whose extracellular domain mediates ligand-binding, resulting in the transmission of developmental signals by the cytoplasmic domain.

In various specific embodiments, *in vitro* assays can be carried out with representative cells of cell types involved in a patient's disorder, to determine if a Therapeutic has a desired effect upon such cell types.

In another embodiment, cells of a patient tissue sample suspected of being pre-neoplastic are similarly plated out or grown *in vitro*, and exposed to a Therapeutic. The Therapeutic which results in a cell phenotype that is more normal (*i.e.*, less representative of a pre-neoplastic state, neoplastic state, malignant state, or transformed phenotype) is selected for therapeutic use. Many assays standard in the art can be used to assess whether a pre-neoplastic state, neoplastic state, or a transformed or malignant phenotype, is present. For example, characteristics associated with a transformed phenotype (a set of *in vitro* characteristics associated with a tumorigenic ability *in vivo*) include a more rounded cell morphology, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, release of proteases such as plasminogen activator, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250,000 dalton surface protein, etc. (see Luria et al., 1978, *General Virology*, 3d Ed., John Wiley & Sons, New York pp. 436-446).

In other specific embodiments, the *in vitro* assays described *supra* can be carried out using a cell line, rather than a cell sample derived from the specific patient to be treated, in which the cell line is derived from or displays characteristic(s) associated with the malignant, neoplastic

or pre-neoplastic disorder desired to be treated or prevented, or is derived from the neural or other cell type upon which an effect is desired, according to the present invention.

The Antagonist Therapeutics are administered therapeutically (including prophylactically): (1) in diseases or disorders involving increased (relative to normal, or desired) levels of Notch or Delta function, for example, where the Notch or Delta protein is overexpressed or overactive; and (2) in diseases or disorders wherein *in vitro* (or *in vivo*) assays indicate the utility of Delta antagonist administration. The increased levels of Notch or Delta function can be readily detected by methods such as those described above, by quantifying protein and/or RNA. *In vitro* assays with cells of patient tissue sample or the appropriate cell line or cell type, to determine therapeutic utility, can be carried out as described above.

5.8.1. MALIGNANCIES

Malignant and pre-neoplastic conditions which can be tested as described *supra* for efficacy of intervention with Antagonist or Agonist Therapeutics, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to those described below in Sections 5.8.1 and 5.9.1.

Malignancies and related disorders, cells of which type can be tested *in vitro* (and/or *in vivo*), and upon observing the appropriate assay result, treated according to the present invention, include but are not limited to those listed in Table 1 (for a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia):

TABLE 1
MALIGNANCIES AND RELATED DISORDERS

- Leukemia
  - acute leukemia
    - acute lymphocytic leukemia
    - acute myelocytic leukemia
      - myeloblastic
      - promyelocytic
      - myelomonocytic
      - monocytic
      - erythroleukemia
  - chronic leukemia
    - chronic myelocytic (granulocytic) leukemia
    - chronic lymphocytic leukemia
- Polycythemia vera
- Lymphoma
  - Hodgkin's disease
  - non-Hodgkin's disease
- Multiple myeloma
- Waldenström's macroglobulinemia
- Heavy chain disease
- Solid tumors
  - sarcomas and carcinomas
    - fibrosarcoma
    - myxosarcoma
    - liposarcoma
    - chondrosarcoma
    - osteogenic sarcoma
    - chordoma
    - angiosarcoma
    - endotheliosarcoma
    - lymphangiosarcoma
    - lymphangioendotheliosarcoma
    - synovioma
    - mesothelioma
    - Ewing's tumor
    - leiomyosarcoma
    - rhabdomyosarcoma
    - colon carcinoma
    - pancreatic cancer
    - breast cancer
    - ovarian cancer
    - prostate cancer
    - squamous cell carcinoma
    - basal cell carcinoma
    - adenocarcinoma
    - sweat gland carcinoma
    - sebaceous gland carcinoma
    - papillary carcinoma
    - papillary adenocarcinomas
    - cystadenocarcinoma
    - medullary carcinoma

bronchogenic carcinoma
renal cell carcinoma
hepatoma
bile duct carcinoma
choriocarcinoma
seminoma
embryonal carcinoma
Wilms' tumor
cervical cancer
testicular tumor
lung carcinoma
small cell lung carcinoma
bladder carcinoma
epithelial carcinoma
glioma
astrocytoma
medulloblastoma
craniopharyngioma
ependymoma
pinealoma
hemangioblastoma
acoustic neuroma
oligodendroglioma
menangioma
melanoma
neuroblastoma
retinoblastoma

In specific embodiments, malignancy or dysproliferative changes (such as metaplasias and dysplasias) are treated or prevented in epithelial tissues such as those in the cervix, esophagus, and lung.

Malignancies of the colon and cervix exhibit increased expression of human Notch relative to such non-malignant tissue (see PCT Publication no. WO 94/07474 published April 14, 1994, incorporated by reference herein in its entirety). Thus, in specific embodiments, malignancies or premalignant changes of the colon or cervix are treated or prevented by administering an effective amount of an Antagonist Therapeutic, *e.g.*, a Delta derivative, that antagonizes Notch function. The presence of increased Notch expression in colon, and cervical cancer suggests that many more cancerous and hyperproliferative conditions exhibit upregulated Notch. Thus, in specific embodiments, various

cancers, e.g., breast cancer, squamous adenocarcinoma, seminoma, melanoma, and lung cancer, and premalignant changes therein, as well as other hyperproliferative disorders, can be treated or prevented by administration of an Antagonist Therapeutic that antagonizes Notch function.

5.8.2. NERVOUS SYSTEM DISORDERS

Nervous system disorders, involving cell types which can be tested as described *supra* for efficacy of intervention with Antagonist or Agonist Therapeutics, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to nervous system injuries, and diseases or disorders which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian patients) according to the invention include but are not limited to the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems:

(i) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries;

(ii) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia;

(iii) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue;

(iv) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an

abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis;

(v) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis;

(vi) lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcchol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration;

(vii) neurological lesions associated with systemic diseases including but not limited to diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis;

(viii) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and

(ix) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including but not limited to multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

Therapeutics which are useful according to the invention for treatment of a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons (see also Section 5.8). For example, and not by way of limitation, Therapeutics which elicit any of the following effects may be useful according to the invention:

(i) increased survival time of neurons in culture;

(ii) increased sprouting of neurons in culture or *in vivo*;

(iii) increased production of a neuron-associated molecule in culture or *in vivo*, *e.g.*, choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or

(iv) decreased symptoms of neuron dysfunction *in vivo*.

Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may be measured by the method set forth in Arakawa et al. (1990, J. Neurosci. 10:3507-3515); increased sprouting of neurons may be detected by methods set forth in Pestronk et al. (1980, Exp. Neurol. 70:65-82) or Brown et al. (1981, Ann. Rev. Neurosci. 4:17-42); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, *e.g.*, weakness, motor neuron conduction velocity, or functional disability.

In a specific embodiments, motor neuron disorders that may be treated according to the invention include but are not limited to disorders such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and including but not limited to

progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

5.8.3. TISSUE REPAIR AND REGENERATION

In another embodiment of the invention, a Therapeutic of the invention is used for promotion of tissue regeneration and repair, including but not limited to treatment of benign dysproliferative disorders. Specific embodiments are directed to treatment of cirrhosis of the liver (a condition in which scarring has overtaken normal liver regeneration processes), treatment of keloid (hypertrophic scar) formation (disfiguring of the skin in which the scarring process interferes with normal renewal), psoriasis (a common skin condition characterized by excessive proliferation of the skin and delay in proper cell fate determination), and baldness (a condition in which terminally differentiated hair follicles (a tissue rich in Notch) fail to function properly). In another embodiment, a Therapeutic of the invention is used to treat degenerative or traumatic disorders of the sensory epithelium of the inner ear.

5.9. PROPHYLACTIC USES

5.9.1. MALIGNANCIES

The Therapeutics of the invention can be administered to prevent progression to a neoplastic or malignant state, including but not limited to those disorders listed in Table 1. Such administration is indicated where the Therapeutic is shown in assays, as described *supra*, to have utility for treatment or prevention of such disorder. Such prophylactic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has

occurred (for review of such abnormal growth conditions, see Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-79.) Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. As but one example, endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

Alternatively or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed phenotype, or of a malignant phenotype, displayed *in vivo* or displayed *in vitro* by a cell sample from a patient, can indicate the desirability of prophylactic/therapeutic administration of a Therapeutic of the invention. As mentioned *supra*, such characteristics of a transformed phenotype include morphology changes, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, protease release, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250,000 dalton cell surface protein, etc. (see also *id.*, at pp. 84-90 for characteristics associated with a transformed or malignant phenotype).

In a specific embodiment, leukoplakia, a benign-appearing hyperplastic or dysplastic lesion of the epithelium, or Bowen's disease, a carcinoma *in situ*, are pre-neoplastic lesions indicative of the desirability of prophylactic intervention.

In another embodiment, fibrocystic disease (cystic hyperplasia, mammary dysplasia, particularly adenosis (benign epithelial hyperplasia)) is indicative of the desirability of prophylactic intervention.

In other embodiments, a patient which exhibits one or more of the following predisposing factors for malignancy is treated by administration of an effective amount of a Therapeutic: a chromosomal translocation associated with a malignancy (*e.g.*, the Philadelphia chromosome for chronic myelogenous leukemia, t(14;18) for follicular lymphoma, etc.), familial polyposis or Gardner's syndrome (possible forerunners of colon cancer), benign monoclonal gammopathy (a possible forerunner of multiple myeloma), and a first degree kinship with persons having a cancer or precancerous disease showing a Mendelian (genetic) inheritance pattern (*e.g.*, familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, medullary thyroid carcinoma with amyloid production and pheochromocytoma, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia, and Bloom's syndrome; *see* Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 112-113) etc.)

In another specific embodiment, an Antagonist Therapeutic of the invention is administered to a human patient to prevent progression to breast, colon, or cervical cancer.

5.9.2. OTHER DISORDERS

In other embodiments, a Therapeutic of the invention can be administered to prevent a nervous system disorder described in Section 5.8.2, or other disorder (*e.g.*, liver cirrhosis, psoriasis, keloids, baldness) described in Section 5.8.3.

5.10. DEMONSTRATION OF THERAPEUTIC OR PROPHYLACTIC UTILITY

The Therapeutics of the invention can be tested *in vivo* for the desired therapeutic or prophylactic activity. For example, such compounds can be tested in suitable animal model systems prior to testing in humans, including but not limited to rats, mice, chicken, cows, monkeys, rabbits, etc. For *in vivo* testing, prior to administration to humans, any animal model system known in the art may be used.

5.11. ANTISENSE REGULATION OF *DELTA* EXPRESSION

The present invention provides the therapeutic or prophylactic use of nucleic acids of at least six nucleotides that are antisense to a gene or cDNA encoding Delta or a portion thereof. "Antisense" as used herein refers to a nucleic acid capable of hybridizing to a portion of a *Delta* RNA (preferably mRNA) by virtue of some sequence complementarity. Such antisense nucleic acids have utility as Antagonist Therapeutics of the invention, and can be used in the treatment or prevention of disorders as described *supra* in Section 5.8 and its subsections.

The antisense nucleic acids of the invention can be oligonucleotides that are double-stranded or single-stranded, RNA or DNA or a modification or derivative thereof, which can be directly administered to a cell, or which can be produced intracellularly by transcription of exogenous, introduced sequences.

In a specific embodiment, the *Delta* antisense nucleic acids provided by the instant invention can be used for the treatment of tumors or other disorders, the cells of

which tumor type or disorder can be demonstrated (*in vitro* or *in vivo*) to express a *Delta* gene or a *Notch* gene. Such demonstration can be by detection of RNA or of protein.

The invention further provides pharmaceutical compositions comprising an effective amount of the *Delta* antisense nucleic acids of the invention in a pharmaceutically acceptable carrier, as described *infra* in Section 5.12. Methods for treatment and prevention of disorders (such as those described in Sections 5.8 and 5.9) comprising administering the pharmaceutical compositions of the invention are also provided.

In another embodiment, the invention is directed to methods for inhibiting the expression of a *Delta* nucleic acid sequence in a prokaryotic or eukaryotic cell comprising providing the cell with an effective amount of a composition comprising an antisense *Delta* nucleic acid of the invention.

Delta antisense nucleic acids and their uses are described in detail below.

5.11.1. *DELTA* ANTISENSE NUCLEIC ACIDS

The *Delta* antisense nucleic acids are of at least six nucleotides and are preferably oligonucleotides (ranging from 6 to about 50 oligonucleotides). In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appending groups such as peptides, or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO 88/09810, published December 15, 1988) or blood-brain barrier (see, *e.g.*, PCT Publication No. WO 89/10134, published April 25, 1988),

hybridization-triggered cleavage agents (see, *e.g.*, Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents (see, *e.g.*, Zon, 1988, Pharm. Res. 5:539-549).

In a preferred aspect of the invention, a *Delta* antisense oligonucleotide is provided, preferably of single-stranded DNA. In a most preferred aspect, such an oligonucleotide comprises a sequence antisense to the sequence encoding an SH3 binding domain or a Notch-binding domain of Delta, most preferably, of human Delta. The oligonucleotide may be modified at any position on its structure with substituents generally known in the art.

The Delta antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

In another embodiment, the oligonucleotide comprises at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the oligonucleotide comprises at least one modified phosphate backbone selected

from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the oligonucleotide is an α-anomeric oligonucleotide. An α-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641).

The oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

Oligonucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

In a specific embodiment, the *Delta* antisense oligonucleotide comprises catalytic RNA, or a ribozyme (see, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al., 1990, Science 247:1222-1225). In another embodiment, the oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

In an alternative embodiment, the *Delta* antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector can be introduced *in vivo* such that it is taken up by a cell, within which cell the vector or a portion thereof is

transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the *Delta* antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the Delta antisense RNA can be by any promoter known in the art to act in mammalian, preferably human, cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980. Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a *Delta* gene, preferably a human *Delta* gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded Delta antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with a *Delta* RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a

tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

5.11.2. THERAPEUTIC UTILITY OF *DELTA* ANTISENSE NUCLEIC ACIDS

The *Delta* antisense nucleic acids can be used to treat (or prevent) malignancies or other disorders, of a cell type which has been shown to express *Delta* or *Notch*. In specific embodiments, the malignancy is cervical, breast, or colon cancer, or squamous adenocarcinoma. Malignant, neoplastic, and pre-neoplastic cells which can be tested for such expression include but are not limited to those described *supra* in Sections 5.8.1 and 5.9.1. In a preferred embodiment, a single-stranded DNA antisense Delta oligonucleotide is used.

Malignant (particularly, tumor) cell types which express *Delta* or *Notch* RNA can be identified by various methods known in the art. Such methods include but are not limited to hybridization with a *Delta* or *Notch*-specific nucleic acid (*e.g.* by Northern hybridization, dot blot hybridization, *in situ* hybridization), observing the ability of RNA from the cell type to be translated *in vitro* into Notch or Delta, immunoassay, etc. In a preferred aspect, primary tumor tissue from a patient can be assayed for Notch or Delta expression prior to treatment, *e.g.*, by immunocytochemistry or *in situ* hybridization.

Pharmaceutical compositions of the invention (see Section 5.12), comprising an effective amount of a *Delta* antisense nucleic acid in a pharmaceutically acceptable carrier, can be administered to a patient having a malignancy which is of a type that expresses *Notch* or *Delta* RNA or protein.

The amount of *Delta* antisense nucleic acid which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. Where possible, it is desirable to determine the

antisense cytotoxicity of the tumor type to be treated *in vitro*, and then in useful animal model systems prior to testing and use in humans.

In a specific embodiment, pharmaceutical compositions comprising *Delta* antisense nucleic acids are administered via liposomes, microparticles, or microcapsules. In various embodiments of the invention, it may be useful to use such compositions to achieve sustained release of the *Delta* antisense nucleic acids. In a specific embodiment, it may be desirable to utilize liposomes targeted via antibodies to specific identifiable tumor antigens (Leonetti et al., 1990, Proc. Natl. Acad. Sci. U.S.A. 87:2448-2451; Renneisen et al., 1990, J. Biol. Chem. 265:16337-16342).

5.12. THERAPEUTIC/PROPHYLACTIC ADMINISTRATION AND COMPOSITIONS

The invention provides methods of treatment (and prophylaxis) by administration to a subject of an effective amount of a Therapeutic of the invention. In a preferred aspect, the Therapeutic is substantially purified. The subject is preferably an animal, including but not limited to animals such as cows, pigs, chickens, etc., and is preferably a mammal, and most preferably human.

Various delivery systems are known and can be used to administer a Therapeutic of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, expression by recombinant cells, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a Therapeutic nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together

with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, *e.g.*, in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

In another embodiment, the Therapeutic can be delivered in a vesicle, in particular a liposome (*see* Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; *see* generally *ibid.*)

In yet another embodiment, the Therapeutic can be delivered in a controlled release system. In one embodiment, a pump may be used (*see* Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (*see* Medical Applications of Controlled Release, Langer and Wise

(eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); *see also* Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, *i*.e., the brain, thus requiring only a fraction of the systemic dose (*see*, *e.g.*, Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

In a specific embodiment where the Therapeutic is a nucleic acid encoding a protein Therapeutic, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see *e.g.*, Joliot et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868), etc. Alternatively, a nucleic acid Therapeutic can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

In specific embodiments directed to treatment or prevention of particular disorders, preferably the following forms of administration are used:

Disorder	Preferred Forms of Administration
Cervical cancer	Topical
Gastrointestinal cancer	Oral; intravenous
Lung cancer	Inhaled; intravenous
Leukemia	Intravenous; extracorporeal
Metastatic carcinomas	Intravenous; oral
Brain cancer	Targeted; intravenous;intrathecal
Liver cirrhosis	Oral; intravenous
Psoriasis	Topical
Keloids	Topical
Baldness	Topical
Spinal cord injury	Targeted; intravenous; intrathecal
Parkinson's disease	Targeted; intravenous; intrathecal
Motor neuron disease	Targeted; intravenous; intrathecal
Alzheimer's disease	Targeted; intravenous; intrathecal

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a Therapeutic, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel,

sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the Therapeutic, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for

injection or saline can be provided so that the ingredients may be mixed prior to administration.

The Therapeutics of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the Therapeutic of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20-500 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental

agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

5.13. DIAGNOSTIC UTILITY

Delta proteins, analogues, derivatives, and subsequences thereof, *Delta* nucleic acids (and sequences complementary thereto), anti-Delta antibodies, have uses in diagnostics. Such molecules can be used in assays, such as immunoassays, to detect, prognose, diagnose, or monitor various conditions, diseases, and disorders affecting Delta expression, or monitor the treatment thereof. In particular, such an immunoassay is carried out by a method comprising contacting a sample derived from a patient with an anti-Delta antibody under conditions such that immunospecific binding can occur, and detecting or measuring the amount of any immunospecific binding by the antibody. In a specific aspect, such binding of antibody, in tissue sections, preferably in conjunction with binding of anti-Notch antibody can be used to detect aberrant Notch and/or Delta localization or aberrant levels of Notch-Delta colocalization in a disease state. In a specific embodiment, antibody to Delta can be used to assay in a patient tissue or serum sample for the presence of Delta where an aberrant level of Delta is an indication of a diseased condition. Aberrant levels of Delta binding ability in an endogenous Notch protein, or aberrant levels of binding ability to Notch (or other Delta ligand) in an endogenous Delta protein may be indicative of a disorder of cell fate (*e.g.*, cancer, etc.) By "aberrant levels," is meant increased or decreased levels relative to that present, or a standard level representing that present, in an analogous sample from a portion of the body or from a subject not having the disorder.

The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays,

ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few.

Delta genes and related nucleic acid sequences and subsequences, including complementary sequences, and other toporythmic gene sequences, can also be used in hybridization assays. *Delta* nucleic acid sequences, or subsequences thereof comprising about at least 8 nucleotides, can be used as hybridization probes. Hybridization assays can be used to detect, prognose, diagnose, or monitor conditions, disorders, or disease states associated with aberrant changes in Delta expression and/or activity as described *supra*. In particular, such a hybridization assay is carried out by a method comprising contacting a sample containing nucleic acid with a nucleic acid probe capable of hybridizing to *Delta* DNA or RNA, under conditions such that hybridization can occur, and detecting or measuring any resulting hybridization.

Additionally, since Delta binds to Notch, Delta or a binding portion thereof can be used to assay for the presence and/or amounts of Notch in a sample, *e.g.*, in screening for malignancies which exhibit increased Notch expression such as colon and cervical cancers.

6. A *DELTA* HOMOLOG IN THE CHICK IS EXPRESSED IN PROSPECTIVE NEURONS

As described herein, we have isolated and characterized a chick *Delta* homologue, *C-Delta-1*. We show that *C-Delta-1* is expressed in prospective neurons during neurogenesis, as new cells are being born and their fates decided. Our data in the chick, suggest that both the Delta/Notch signalling mechanism and its role in neurogenesis have been conserved in vertebrates.

6.1. CLONING OF C-DELTA-1

We identified a chick *Delta* homologue, *C-Delta-1*, using the polymerase chain reaction (PCR) and degenerate oligonucleotide primers (Figures 1a, 1b and 2, SEQ ID NOS:1, 2, 3 and 4). *C-Delta-1* was cloned by PCR using the degenerate oligonucleotide primers TTCGGITT(C/T)ACITGGCCIGGIAC (SEQ ID NO:19) and TCIATGCAIGTICCICC(A/G)TT (SEQ ID NO:20) which correspond to the fly Delta protein sequences FGFTWPGT (SEQ ID NO:21) and NGGTCID (SEQ ID NO:22), respectively (Vässin et al., 1987, EMBO J. 6:3431-3440; Kopczynski et al., 1988, Genes Dev. 2:1723-1735). The initial reaction used 50ng of first-strand oligo-d(T)-primed cDNA from stage 4-6 embryos, 1μg of each primer, 0.2mM dNTPs, 2U. of Taq polymerase, in 50μl of the supplied buffer (Perkin-Elmer). 40 cycles of amplification were performed at 94°C/30sec; 50°C/2min; 72°C/2min. Amplified DNA fragments were separated on an agarose gel, cloned in Bluescript pKS⁻ (Stratagene) and sequenced. Two *Delta* homologs were identified, one of which (*C-Delta-1*) is expressed in the nervous system. Of the homolog that is expressed in the nervous system, two variants were identified that differ at the carboxy-terminal end of the encoded protein due to an alternative splicing event at the 3' end of the C-*Delta*-1 gene. One encoded protein has 12 extra amino acids at the carboxy-terminal end, relative to the other encoded protein. The sequence of the shorter encoded variant is set forth in SEQ ID NO:2. The longer variant encoded by SEQ ID NO:3 and identified by the amino acid sequence of SEQ ID NO:4, consists of the amino acid sequence of SEQ ID NO:2 plus twelve additional amino acids at the 3' end (SIPPGSRTSLGV). The longer variant was used in the experiments described below. When tested for biological activity by injection of RNA into *Xenopus* oocytes, each of the variants had the same biological activity.

DNA fragments corresponding to *C-Delta-1* were used to screen a stage 17 spinal cord cDNA library and several full-length clones were obtained and sequenced. We amplified

DNA fragments from chick *C-Notch-1* gene by similar methods (data not shown); partial sequence data and pattern of expression indicate close similarity to the rodent Notch-1 gene (Weinmaster et al., 1991, Development 113:199-205; Weinmaster et al., 1992, Development 116:931-941; Lardelli & Lendahl, 1993, Exp. Cell Res. 204:364-372). Sequences were analyzed using the Wisconsin GCG set of programs. The GenBank Accession number for the Chick Delta-1 mRNA is U26590. The DNA sequence of *C-Delta-1* corresponds to a protein of 722 amino acids, structurally homologous to *Drosophila* Delta (Figs. 3, 4) and clearly distinct from vertebrate homologs of the Delta-related Serrate protein, which we have also cloned (data not shown). C-Delta-1 contains a putative transmembrane domain, a signal sequence and 8 EGF-like repeats in its extracellular region (one repeat less than *Drosophila* Delta). The amino-terminal domain of C-Delta-1 is closely related to a similar domain in the fly Delta protein, described as necessary and sufficient for *in vitro* binding to Notch (Muskavitch, 1994, Dev. Biol. 166:415-430). This conserved region includes the so-called DSL motif (Fig. 4) (Henderson et al., 1994, Development 120:2913-2924; Tax et al., 1994, Nature 368:150-154), shared by all known members of the family of presumed ligands of Notch-like proteins (Delta and Serrate in *Drosophila*; Lag-2 and Apx-1 in *Caenorhabditis elegans*) (Henderson et al., 1994, Development 120:2913-2924; Tax et al., 1994, Nature 368:150-154; Fleming et al., 1990, Genes Dev. 4:2188-2201; Thomas et al., 1991, Development 111:749-761; Mello et al., 1994, Cell 77:95-106). A second cysteine-rich N-terminal region is conserved between the fly and chick proteins, but absent from the related *C. elegans* proteins (Fig. 4). The *Xenopus Delta-1* homologue, *X-Delta-1* which encodes a protein that is 81% identical to C-Delta-1 and shows all the above structural motifs (Fig. 3), has also been cloned. The structural conservation between the chick and fly Delta proteins, including domains identified as critical for Notch binding (Muskavitch, 1994, Dev. Biol. 166:415-430), suggests

that C-Delta-1 functions as a ligand for a chick Notch protein, and that a Delta/Notch-mediated mechanism of lateral inhibition might operate in the chick.

6.2. C-DELTA-1 AND C-NOTCH-1 EXPRESSION CORRELATES WITH ONSET OF NEUROGENESIS

During *Drosophila* neurogenesis, *Delta* is transiently expressed in neural precursors, inhibiting neighboring *Notch*-expressing cells from also becoming neural (Haenlin et al., 1990, Development 110:905-914; Kooh et al., 1993, Development 117:493-507). If *C-Delta-1* acts similarly during chick neurogenesis, it should also be transiently expressed in neuronal precursor cells, while these are becoming determined. An analysis of *C-Delta-1* expression in the developing CNS indicates that this is indeed the case.

In summary, wholemount *in situ* hybridization was performed. Formaldehyde fixed embryos were treated with protease and refixed with 4% formaldehyde/0.1% glutaraldehyde. Hybridization with DIG-labelled RNA probes was performed under stringent conditions (1.3xSSC, 50% formamide, 65°C, pH5) in a buffer containing 0.2% Tween-20 and 0.5% CHAPS. Washed embryos were treated with Boehringer Blocking Reagent and incubated overnight in alkaline phosphatase-coupled anti-DIG antibody. After extensive washes, embryos were stained from 30min to overnight. The embryo in Figure 5e was wax-sectioned after hybridization.

C-Delta-1 expression in the neural plate is first detected at stage 6-7 (31h, 0/1 somite), in scattered cells just anterior to the presomitic mesoderm (Fig. 5*b*, 5*c*). This region gives rise to the mid/posterior hindbrain, where the earliest differentiated CNS neurons are first detected by a neurofilament antibody at stage 9 (31h, 7-9 somites) (Sechrist & Bronner-Fraser, 1991, Neuron 7:947-963), 6h after the initial *C-Delta-1* expression (Table 2).

TABLE 2

Neural tube domains	Hamburger-Hamilton Stage (nominal age in h; somite nos.)		
	End final S-phase	Initial *C-Delta-1* expression	Initial NF expression
Mid/posterior Hindbrain	4 (19h; 0)	6 (24h; 0)	9 (31h; 7-9)
Spinal cord, somites 5-8	6 (24h; 0)	8 (28h; 4-6)	10 (36h; 10-12)
Forebrain/ Midbrain	7 (25h; 1-3)	8 (28h; 4-6)	10 (36h; 10-12)
Spinal cord, somites 9-12	8 (28h; 4-6)	9 (31h; 7-9)	11 (43h; 13-15)

As neurogenesis proceeds, expression of *C-Delta-1* continues to foreshadow the spatio-temporal pattern of neuronal differentiation (Table 2), spreading posteriorly along the spinal cord and anteriorly into the midbrain and forebrain (Fig 5*d*, 5*e*). For example, the most posterior expressing cells in the stage 8 spinal cord are at the level of the prospective 6th somite, 6-8h before the first neurons at that level express neurofilament antigen (Sechrist & Bronner-Fraser, 1991, Neuron 7:947-963) (Table 2). Table 2 shows that the appearance of *C-Delta-1* expression closely follows the withdrawal of the first neuronal precursors from the division cycle and precedes the appearance of neurofilament (NF) antigen in the resultant differentiating neurons. Mid-hindbrain comprises rhombomeres 4-6, the level of the otic primordium; posterior hindbrain includes rhombomeres 7 and 8, and somites 1-4. Data for the timing of withdrawal from cell-division and for neurofilament expression are taken from Sechrist et al., 1991, Neuron 7:947-963. In all cases, *C-Delta-1* is expressed in scattered cells within domains of uniform *C-Notch-1* expression (Fig. 5*a*).

6.3. LOCALIZATION AND TIME-COURSE EXPRESSION OF C-DELTA-1

The localization and time-course of *C-Delta-1* expression indicate that the gene is switched on at an early step in neurogenesis, and that the cells expressing *C-Delta-1* are prospective neurons that have not yet begun to display differentiation markers. To test this hypothesis, we made use of the observations of Sechrist and Bronner-Fraser (Sechrist & Bronner-Fraser, 1991, Neuron 7:947-963) that prospective neurons are the only non-cycling cells in the early neural tube. They finish their final S phase 11-15h before expressing neurofilament antigen (Table 2) and their nuclei, after completing a last mitosis, adopt a characteristic location near the basal surface of the neuroepithelium, where all the other cell nuclei are in S-phase (Sechrist & Bronner-Fraser, 1991, Neuron 7:947-963; Martin & Langman, 1965, J. Embryol. Exp. Morphol. 14:23-35) (Fig. 6a). We labelled stage 7-9 embryos with bromodeoxyuridine (BrdU), and double-stained for BrdU incorporation and *C-Delta-1* expression. 95% of the *C-Delta-1*-expressing cells were unlabelled, with their nuclei predominantly located near the basal surface, where most other nuclei were BrdU-labelled (Fig. 6b, 6c). 75µl 0.1mM BrdU in PBS was dropped onto stage 7-9 embryos which were incubated at 38°C for 2-4h before fixation for *in situ* hybridization. 15µm cryostat sections were hybridized with DIG-labelled RNA probes, essentially according to the method of Strähle *et al.* (Strähle et al., 1994, Trends In Genet. Sci. 10:75-76). After staining, slides were washed in PBS, and processed for BrdU immunodetection (Biffo et al., 1992, Histochem. Cytochem. 40:535-540). Anti-BrdU (1:1000; Sigma) was detected using FITC-coupled goat anti-mouse secondary antibody (Cappel). *C-Delta-1* expression was examined by DIC microscopy, and BrdU-labelling by conventional and confocal fluorescence microscopy. These results imply that *C-Delta-1* is expressed in cells that have withdrawn from the cell cycle and must indeed be prospective neurons. The few BrdU+/*C-*

Delta-1⁻ cells have their nuclei outside the basal zone; these may be cells that finished their final S-phase soon after exposure to BrdU, moved apically to complete their final mitosis, and switched on *C-Delta-1* expression. *C-Delta-1* is also expressed in the later neural tube and peripheral nervous system. Again, the timing of expression and the location of the expressing cells imply that they are neuronal precursors that have not yet begun to differentiate (data not shown). Thus, *C-Delta-1* expression appears to be the earliest known marker for prospective neurons.

In addition, the transcription pattern of both C-Delta-1 and C-Serrate-1 overlap that of C-Notch-1 in many regions of the embryo (data not shown) which suggest that C-Notch-1, like Notch in *Drosophila*, is a receptor for both proteins. In particular, all three genes are expressed in the neurogenic region of the developing central nervous system, and here a striking relationship is seen: the expression of both C-Serrate-1 and C-Delta-1 is confined to the domain of C-Notch-1 expression; but within this domain, the regions of C-Serrate-1 and C-Delta-1 are precisely complementary. The overlapping expression patterns suggest conservation of their functional relationship with Notch and imply that development of the chick and in particular the central nervous system involves the concerted interaction of C-Notch-1 with different ligands at different locations.

6.4. DISCUSSION

The *Xenopus* homolog of C-Delta-1 has been cloned in a similar manner. In brief, a PCR fragment of X-Delta-1 was isolated and sequenced. This fragment was then used to identify the full length clone of X-Delta-1. The X-Delta-1 expression pattern was studied. It was shown that X-Delta-1 is expressed in scattered cells in the domain of the neural plate where primary neuronal precursors are being generated, suggesting that the cells expressing X-Delta-1 are the prospective primary neurons. In addition, X-Delta-1 is also expressed at other sites and times of neurogenesis, including

the anterior neural plate and neurogenic placodes and later stages of neural tube development when secondary neurons are generated. Ectopic *X-Delta-1* activity inhibited production of primary neurons; interference with endogenous *X-Delta-1* activity resulted in overproduction of primary neurons. These results show that X-Delta-1 mediates lateral inhibition delivered by prospective neurons to adjacent cells. It was shown that ectopic expression of X-Delta-1 in *Xenopus* eggs suppresses primary neurogenesis, and that ectopic expression of a truncated X-Delta-1 protein which retains only two amino acids of the cytoplasmic domain interferes with endogenous signalling and leads to extra cells developing as neuronal precursors. (Chitnis et al., Nature (in press). Preliminary evidence indicates that *C-Delta-1* has a similar inhibitory action when expressed in *Xenopus* embryos (data not shown). We propose that *C-Delta-1*, like its *Drosophila* and *Xenopus* counterparts, mediates lateral inhibition throughout neurogenesis to restrict the proportion of cells that, at any time, become committed to a neural fate. C-Delta-1 is generally expressed during neurogenesis in many other sites, in both the CNS and PNS, and, for example, the developing ear. It has been shown in the CNS that C-Notch is expressed in the ventricular zone of the E5 chick hindbrain, in dividing cells adjacent to the lumen of the neural tube. C-Delta-1 is expressed in the adjacent layer of cells, which have stopped dividing and are becoming committed as neuronal precursor cells. Thus, Delta/Notch signalling could act here, as in other neural tissues, to maintain a population of uncommitted cycling neuronal stem cells.

7. ISOLATION AND CHARACTERIZATION OF A MOUSE *DELTA* HOMOLOG

A mouse *Delta* homolog, termed M-*Delta*-1, was isolated as follows:

Mouse *Delta*-1 gene

Tissue Origin: 8.5 and 9.5-day mouse embryonic RNA

Isolation Method:

a) random primed cDNA against above RNA

b) PCR of above cDNA using

PCR primer 1: GGITTCACITGGCCIGGIACNTT (SEQ ID NO:23) [encoding GFTWPGTF (SEQ ID NO:24), a region which is specific for Delta-, not Serrate-like proteins]

PCR primer 2: GTICCICC(G/A)TT(C/T)TT(G/A)CAIGG(G/A)TT (SEQ ID NO:25) [encoding NPCKNGGT (SEQ ID NO:26), a sequence present in many of the EGF-like repeats]

Amplification conditions: 50 ng cDNA, 1 μg each primer, 0.2 mM dNTP's, 1.8 U Taq (Perkin-Elmer) in 50 μl of supplied buffer. 40 cycles of: 94°C/30 sec, 45°C/2 min, 72°C/1 min extended by 2 sec each cycle.

The amplified fragment was an approximately 650 base pair fragment which was partially sequenced to determine its relationship to *C-Delta-1*.

c) a mouse 11.5 day cDNA library (Clontech) was screened. Of several positive clones, one (*pMDL2*; insert size approximately 4 kb) included the complete protein-coding region whose DNA sequence was completely determined.

Figure 7 (SEQ ID NO:11) shows the nucleotide sequence of the isolated clone containing M-*Delta*-1 DNA.

Figure 8 (SEQ ID NO:12) shows the predicted amino acid sequence of M-Delta-1.

Figure 9 shows and amino acid alignment of the predicted amino acid sequences for M-Delta-1 and C-Delta-1. Identical amino acids are boxed showing the extensive sequence homology. The consensus sequence is shown below (SEQ ID NO:13).

Expression pattern: The expression pattern was determined to be essentially the same as that observed for C-Delta-1, in particular, in the presomitic mesoderm, central nervous system, peripheral nervous system, and kidney.

8. ISOLATION AND CHARACTERIZATION OF A HUMAN DELTA HOMOLOG

A human Delta-1 homolog, termed H-Delta-1 (HDl), was isolated as follows:

A human genomic library with inserts ranging in size from 100-150 kb was probed with an EcoRI fragment of the mouse Delta-1 (M-*Delta*-1) gene. From the library a genomic human PAC clone was isolated which hybridized to the EcoRI fragment. Next, two degenerate oligonucleotides were used to amplify by PCR a fragment of the genomic human PAC clone. The degenerate oligos were:

5' ACIATGAA(C/T)AA(C/T)CTIGCIAA(C/T)TG (SEQ ID NO:27) [encoding TMNNLANC (SEQ ID NO:28)] and

3' AC(A/G)TAIACIGA(C/T)TG(A/G)TA(C/T)TTIGT (SEQ ID NO:29) [encoding TKYQSVYV (SEQ ID NO:30) or

3' GC(A/G/T)ATIAC(A/G)CA(C/T)TC(A/G)TC(C/T)TT(C/T)TC (SEQ ID NO:31) [encoding EKDECVIA (SEQ ID NO:32).

On the basis of the cDNA sequences for chicken and mouse Delta-1, it was expected that fragments of approximately 354 and 387 base pairs would be isolated, using the 5' and the two different 3' oligos, respectively. In fact, however, two single isolates of 525 base pairs and another that was 30 base pairs smaller, as expected, were obtained. The larger isolate was sequenced by dideoxy sequencing. The nucleotide sequence is shown in Figure 10 (SEQ ID NO:14). Also shown in Figure 10 are the predicted amino acid sequences of the amplified DNA fragment (SEQ ID NOS:15, 16, 17) for the three different readings frames. Due to sequencing errors, the full uninterrupted sequence between both primers was not identified. As a consequence, one cannot predict the amino acid sequence directly from the DNA sequence obtained. However, Figure 11 shows the amino acid sequence homology between human Delta-1 (top line) (SEQ ID NO:18) and chick Delta-1 (bottom line) as determined by eye. Because of the sequencing errors, the homology was obtained by switching amongst the three different reading frames to identify the homologous regions.

Using the larger isolate (SEQ ID NO:14) as probe, a human fetal brain plasmid library (Clontech) was screened in an attempt to isolate full-length H-Delta-1 (HD1) genes. This yielded four positive plaques. Two of these positives (HD13 and HD124) survived rescreening and reacted positively with a large human genomic fragment on a Southern Blot. These positive clones were subcloned by digesting with *Eco*RI and ligating the fragments into a Bluescript KS$^-$ vector. The nucleotide sequences of the inserts were obtained by dideoxy sequencing using T3 and T7 primers. The results showed that HD124 was homologous to chicken Delta-1 at both ends; however, one end of HD13 showed no homology. Restriction digestions with a panel of enzymes showed very similar patterns between the two clones, each of which had an insert of about 2 kb, but with differences at the 3′ end of HD13.

HD13 and HD124 were cut with *Bst*XI, *Xba*I, *Hind*III and *Xho*I and the restriction fragments were inserted into Bluescript KS$^-$, and then sequenced as described above to obtain internal sequence. The sequence that was obtained represents the 3′ about 2000 bases of HD1, extending into the 3′ non-coding region. HD13 is contained within HD124; however, the added sequence at the 5′ end of HD13 is likely due to a cloning artifact.

Since the sequence thus obtained did not contain the 5′ end of HD1, HD124 was used as a probe for subsequent hybridizations in a T cell library and in another fetal brain library (Lambda-Zap, Stratagene). A screen of the T cell library resulted in no positives. However, screening the Lambda-Zap library resulted in two positive clones, HD113 and HD118. These clones were inserted into a Bluescript KS$^-$ vector using *Eco*RI as described above. The inserts were digested with a panel of restriction enzymes for comparison with HD13 and HD124, and the 5′ and 3′ ends were sequenced using T3 and T7 primers. HD113 was determined to be only a small piece of cDNA that when sequenced showed no homology to any known Delta. However, HD118 was 3 kb in length, and included the entire sequence of HD124 with additional 5′

sequences. A set of clones were isolated using nested deletions from HDl18; these clones were then subjected to dideoxy sequencing using an automated sequencer. Figure 12A presents the partial nucleotide contig sequence (SEQ ID NO:33) of human *Delta* obtained from clone HDl18. Due to sequencing errors, the full uninterrupted nucleotide sequence of human *Delta* was not determined. Figure 12B shows the partial nucleotide contig sequence (SEQ ID NO:33) of human *Delta* (top line), with the predicted amino acid sequence in three different reading frames presented below, the second line being reading frame 1 (SEQ ID NO:34), the third line being reading frame 2 (SEQ ID NO:35), and the fourth line being reading frame 3 (SEQ ID NO:36).

Sequence homology was determined by eye using the mouse Delta-1 amino acid sequence. The sequences with the greatest degree of homology to the mouse amino acid sequence are boxed in Figure 12B, and represent the predicted amino acid sequence of human Delta-1. The composite resulting amino acid sequence is shown in Figure 14. (In Figure 14, the various uninterrupted portions of the human Delta sequence are assigned respectively, SEQ ID NOS:39 through 65.) Note that due to sequencing errors, the reading frame with the greatest homology is not the same throughout the sequence and shifts at positions where there are errors in the sequence.

Further, the homology determined by eye to chicken and mouse Delta indicates that the amino acid sequence deduced from the determined human *Delta* nucleotide sequence contains all but about the N-terminal 100-150 amino acids of human Delta-1.

Figure 13 presents the nucleotide sequence of mouse *Delta*-1 (top line, SEQ ID NO:37) and the contig nucleotide sequence of human *Delta*-1 as depicted in Figures 12A and 12B (second line, SEQ ID NO:33) and the nucleotide consensus sequence between mouse and human *Delta* (third line, SEQ ID NO:38).

Using probes containing the human Delta 5′ nucleotide sequences presented in Figure 12A, cDNA libraries are probed to isolate the 5′ end of the human Delta gene. Primary positive clones are obtained and then confirmed as secondary positives. The secondary positives are purified and grown further. The DNA is then isolated and subcloned for sequencing.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. A purified vertebrate Delta protein.

2. The protein of claim 1 which is a human protein.

3. The protein of claim 1 which is a mammalian protein.

4. The protein of claim 1 which comprises the amino acid sequence substantially as set forth in amino acid numbers 1-722 of SEQ ID NO:12.

5. A purified derivative or analog of the protein of claim 1, which is able to display one or more functional activities of a Delta protein.

6. A purified derivative or analog of the protein of claim 2, which is able to display one or more functional activities of a human or *D. melanogaster* Delta protein.

7. The derivative or analog of claim 5 which is able to be bound by an antibody directed against a human or *D. melanogaster* Delta protein.

8. A purified fragment of the protein of claim 2, which is able to be bound by an antibody directed against a human Delta protein.

9. A molecule comprising the fragment of claim 8.

10. A purified fragment of the protein of claim 2 which is able to display one or more functional activities of a human Delta protein.

11. A purified fragment of a vertebrate Delta protein comprising a domain of the protein selected from the group consisting of the extracellular domain, DSL domain, domain amino-terminal to the DSL domain, epidermal growth factor-like repeat domain, transmembrane domain, and intracellular domain.

12. A purified fragment of a Delta protein comprising the membrane-associated region of the protein.

13. A purified fragment of a Delta protein comprising an epidermal growth factor-homologous repeat of the protein.

14. The fragment of claim 11 in which the Delta protein is a human Delta protein.

15. A purified fragment of a vertebrate Delta protein comprising a region homologous to a Notch protein or a Delta protein, and consisting of at least six amino acids.

16. A purified fragment of a vertebrate Delta protein comprising the region of the protein with the greatest homology over an identical number of amino acids to amino acid numbers 1-722 as shown in Figure 8 (SEQ ID NO:12).

17. A chimeric protein comprising a fragment of a vertebrate Delta protein consisting of at least 20 amino acids fused via a covalent bond to an amino acid sequence of a second protein, in which the second protein is not the Delta protein.

18. The chimeric protein of claim 17 in which the fragment of a vertebrate Delta protein is a fragment capable of being bound by an anti-Delta antibody.

19. The chimeric protein of claim 18 in which the Delta protein is a human protein.

20. The chimeric protein of claim 19 which is able to display one or more functional activities of a Delta protein.

21. A purified fragment of a vertebrate Delta protein which (a) is capable of being bound by an anti-Delta antibody; and (b) lacks the transmembrane and intracellular domains of the protein.

22. A purified fragment of a vertebrate Delta protein which (a) is capable of being bound by an anti-Delta antibody; and (b) lacks the extracellular domain of the protein.

23. A purified fragment of a vertebrate Delta protein which is able to bind to a Notch protein.

24. The fragment of claim 23, which lacks the epidermal growth factor-like repeats of the Delta protein.

25. The fragment of claim 23 in which the Delta protein is a human Delta protein.

26. The fragment of claim 23, which is a fragment of SEQ ID NO:18.

27. A molecule comprising the fragment of claim 23.

28. The fragment of claim 11 or 21 in which the Delta protein is a human Delta protein.

29. An antibody which is capable of binding the Delta protein of claim 1, and which does not bind to a *Drosophila* Delta protein.

30. An antibody which is capable of binding the Delta protein of claim 2, and which does not bind to a *Drosophila* Delta protein.

31. The antibody of claim 1 which is monoclonal.

32. A molecule comprising a fragment of the antibody of claim 31, which fragment is capable of binding a Delta protein.

33. An isolated nucleic acid comprising a nucleotide sequence encoding a vertebrate Delta protein.

34. The nucleic acid of claim 33 which is DNA.

35. An isolated nucleic acid comprising a nucleotide sequence complementary to the nucleotide sequence of claim 33.

36. An isolated nucleic acid comprising a nucleotide sequence encoding the Delta protein of claim 2.

37. An isolated nucleic acid comprising a fragment of a vertebrate *Delta* gene consisting of at least 50 nucleotides.

38. An isolated nucleic acid comprising a nucleotide sequence encoding the fragment of claim 10.

39. An isolated nucleic acid comprising a nucleotide sequence encoding the fragment of claim 11.

40. An isolated nucleic acid comprising a nucleotide sequence encoding the fragment of claim 23.

41. An isolated nucleic acid comprising a nucleotide sequence encoding a protein, said protein comprising amino acid numbers 1-175 of the human Delta sequence depicted in Figure 11 (SEQ ID NO:18).

42. An isolated nucleic acid comprising a nucleotide sequence encoding the protein of claim 17.

43. A recombinant cell containing the nucleic acid of claim 33.

44. A recombinant cell containing the nucleic acid of claim 39.

45. A recombinant cell containing the nucleic acid of claim 41.

46. A method of producing a vertebrate Delta protein comprising growing a recombinant cell containing the nucleic acid of claim 33 such that the encoded vertebrate Delta protein is expressed by the cell, and recovering the expressed Delta protein.

47. A method of producing a vertebrate Delta protein comprising growing a recombinant cell containing the nucleic acid of claim 41 such that the encoded Delta protein is expressed by the cell, and recovering the expressed Delta protein.

48. A method of producing a protein comprising a fragment of a vertebrate Delta protein, which method comprises growing a recombinant cell containing the nucleic acid of claim 39 such that the encoded protein is expressed by the cell, and recovering the expressed protein.

49. The product of the process of claim 46.

50. The product of the process of claim 47.

51. The product of the process of claim 48.

52. A pharmaceutical composition comprising a therapeutically effective amount of a Delta protein; and a pharmaceutically acceptable carrier.

53. The composition of claim 52 in which the Delta protein is a human Delta protein.

54. A pharmaceutical composition comprising a therapeutically effective amount of the fragment of claim 11; and a pharmaceutically acceptable carrier.

55. A pharmaceutical composition comprising a therapeutically effective amount of the fragment of claim 23; and a pharmaceutically acceptable carrier.

56. A pharmaceutical composition comprising a therapeutically effective amount of a derivative or analog of a Delta protein, which derivative or analog is characterized by the ability to bind to a Notch protein or to a molecule comprising the epidermal growth factor-like repeats 11 and 12 of a Notch protein; and a pharmaceutically acceptable carrier.

57. A pharmaceutical composition comprising a therapeutically effective amount of the nucleic acid of claim 33; and a pharmaceutically acceptable carrier.

58. A pharmaceutical composition comprising a therapeutically effective amount of the nucleic acid of claim 35; and a pharmaceutically acceptable carrier.

59. A pharmaceutical composition comprising a therapeutically effective amount of the nucleic acid of claim 39; and a pharmaceutically acceptable carrier.

60. A pharmaceutical composition comprising a therapeutically effective amount of an antibody which binds to a Delta protein; and a pharmaceutically acceptable carrier.

61. A pharmaceutical composition comprising a therapeutically effective amount of a fragment or derivative of an antibody to a Delta protein containing the binding domain of the antibody; and a pharmaceutically acceptable carrier.

62. A method of treating or preventing a disease or disorder in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a Delta protein or derivative thereof which is able to bind to a Notch protein.

63. The method according to claim 62 in which the disease or disorder is a malignancy characterized by increased Notch activity or increased expression of a Notch protein or of a Notch derivative capable of being bound by an anti-Notch antibody, relative to said Notch activity or expression in an analogous non-malignant sample.

64. The method according to claim 62 in which the disease or disorder is selected from the group consisting of cervical cancer, breast cancer, colon cancer, melanoma, seminoma, and lung cancer.

65. The method according to claim 62 in which the subject is a human.

66. A method of treating or preventing a disease or disorder in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule, in which the molecule is an oligonucleotide which (a) consists of at least six nucleotides; (b) comprises a sequence complementary to at least a portion of an RNA transcript of a *Delta* gene; and (c) is hybridizable to the RNA transcript.

67. A method of treating or preventing a disease or disorder in a subject comprising administering to a subject in which such treatment or prevention is desired an effective amount of the nucleic acid of claim 33 or 39.

68. A method of treating or preventing a disease or disorder in a subject comprising administering to a subject in which such treatment or prevention is desired an effective amount of the antibody of claim 30.

69. The method according to claim 62 in which the disease or disorder is a disease or disorder of the central nervous system.

70. An isolated oligonucleotide consisting of at least six nucleotides, and comprising a sequence complementary to at least a portion of an RNA transcript of a *Delta* gene, which oligonucleotide is hybridizable to the RNA transcript.

71. A pharmaceutical composition comprising the oligonucleotide of claim 70; and a pharmaceutically acceptable carrier.

72. A method of inhibiting the expression of a nucleic acid sequence encoding a Delta protein in a cell comprising providing the cell with an effective amount of the oligonucleotide of claim 70.

73. A method of diagnosing a disease or disorder characterized by an aberrant level of Notch-Delta protein binding activity in a patient, comprising measuring the ability of a Notch protein in a sample derived from the patient to bind to a Delta protein, in which an increase or decrease in the ability of the Notch protein to bind to the Delta protein, relative to the ability found in an analogous sample from a normal individual, indicates the presence of the disease or disorder in the patient.

74. A method of diagnosing a disease or disorder characterized by an aberrant level of Delta protein in a patient, comprising measuring the level of Delta protein in a sample derived from the patient, in which an increase or decrease in the level of Delta protein, relative to the level of Delta protein found in an analogous sample from a normal individual, indicates the presence of the disease or disorder in the patient.

75. A purified human protein which is encoded by a first nucleic acid that is hybridizable to a second nucleic acid having the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33) or having an at least 50 nucleotide portion of said sequence.

76. The fragment of claim 8 which is encoded by a first nucleic acid that is hybridizable to a second nucleic acid having the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33) or having an at least 50 nucleotide portion of said sequence.

77. The fragment of claim 10 which is encoded by a first nucleic acid that is hybridizable to a second nucleic acid having the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33) or having an at least 50 nucleotide portion of said sequence.

78. The fragment of claim 14 which is encoded by a first nucleic acid that is hybridizable to a second nucleic acid having the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33) or having an at least 50 nucleotide portion of said sequence.

79. The fragment of claim 25 which is encoded by a first nucleic acid that is hybridizable to a second nucleic acid having the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33) or having an at least 50 nucleotide portion of said sequence.

80. The fragment of claim 10 or 25, which is a fragment of SEQ ID NO:39.

81. The fragment of claim 28 which is encoded by a first nucleic acid that is hybridizable to a second nucleic acid having the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33) or having an at least 50 nucleotide portion of said sequence.

82. An isolated nucleic acid comprising the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33).

83. An isolated nucleic acid comprising a nucleotide sequence complementary to the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33).

84. A purified protein comprising at least a portion of a human Delta amino acid sequence, said portion selected from the group consisting of amino acid numbers 1-192 depicted in Figure 14 (SEQ ID NO:39), amino acid numbers 205-213 depicted in Figure 14 (SEQ ID NO:43), amino acid numbers 214-370 depicted in Figure 14 (SEQ ID NO:44), amino acid numbers 371-382 depicted in Figure 14 (SEQ ID NO:45), amino acid numbers 394-418 depicted in Figure 14 (SEQ ID NO:49), amino acid numbers 419-428 depicted in Figure 14 (SEQ

ID NO:50), amino acid numbers 443-458 depicted in Figure 14 (SEQ ID NO:52), amino acid numbers 459-469 depicted in Figure 14 (SEQ ID NO:53), amino acid numbers 470-495 depicted in Figure 14 (SEQ ID NO:54), amino acid numbers 496-508 depicted in Figure 14 (SEQ ID NO:55), and amino acid numbers 516-519 depicted in Figure 14 (SEQ ID NO:59).

85. The protein of claim 84 which is encoded by a first nucleic acid that is hybridizable to a second nucleic acid having the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33) or having an at least 50 nucleotide portion of said sequence.

86. A purified protein which is encoded by a first nucleic acid hybridizable under stringent conditions to a second nucleic acid having a nucleotide sequence comprising a sequence selected from the group consisting of nucleotide numbers 60-634 depicted in Figure 12B (SEQ ID NO:33), nucleotide numbers 746-772 depicted in Figure 12B (SEQ ID NO:33), nucleotide numbers 775-1245 depicted in Figure 12B (SEQ ID NO:33), nucleotide numbers 1249-1284 depicted in Figure 12B (SEQ ID NO:33), nucleotide numbers 1415-1489 depicted in Figure 12B (SEQ ID NO:33), nucleotide numbers 1493-1522 depicted in Figure 12B (SEQ ID NO:33), nucleotide numbers 1526-1567 depicted in Figure 12B (SEQ ID NO:33), nucleotide numbers 1570-1618 depicted in Figure 12B (SEQ ID NO:33), nucleotide numbers 1622-1653 depicted in Figure 12B (SEQ ID NO:33), nucleotide numbers 1658-1735 depicted in Figure 12B (SEQ ID NO:33), and nucleotide numbers 1739-1777 depicted in Figure 12B (SEQ ID NO:33).

87. The protein of claim 2 which comprises a portion of the human Delta amino acid sequence set forth in Figure 14, said portion selected from the group consisting of amino acid numbers 1-192 (SEQ ID NO:39), amino acid numbers 205-213 14 (SEQ ID NO:43), amino acid numbers 214-370 (SEQ ID NO:44), amino acid numbers 371-382 (SEQ ID NO:45), amino acid

numbers 394-418 (SEQ ID NO:49), amino acid numbers 419-428 (SEQ ID NO:50), amino acid numbers 443-458 (SEQ ID NO:52), amino acid numbers 459-469 (SEQ ID NO:53), amino acid numbers 470-495 (SEQ ID NO:54), amino acid numbers 496-508 (SEQ ID NO:55), and amino acid numbers 516-519 (SEQ ID NO:59).

88. The protein of claim 75 in which the first nucleic acid is hybridizable to the second nucleic acid under conditions of high stringency.

89. The fragment of claim 76, 77 or 78 in which the first nucleic acid is hybridizable to the second nucleic acid under conditions of high stringency.

90. An isolated nucleic acid hybridizable under conditions of high stringency to a nucleic acid having the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33) or having an at least 50 nucleotide portion of said sequence.

91. The nucleic acid of claim 90 which comprises a cDNA sequence hybridizable under conditions of high stringency to a nucleic acid having the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33) or having an at least 50 nucleotide portion of said sequence.

92. An isolated nucleic acid comprising a nucleotide sequence complementary to a cDNA sequence hybridizable under conditions of high stringency to a nucleic acid having the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33) or having an at least 50 nucleotide portion of said sequence.

93. A purified human protein which is encoded by a first nucleic acid that is hybridizable to a second nucleic acid having the nucleotide sequence depicted in Figure 10 (SEQ ID NO:14) or having an at least 50 nucleotide portion of said sequence.

94. The fragment of claim 8 which is encoded by a first nucleic acid that is hybridizable to a second nucleic acid having the nucleotide sequence depicted in Figure 10 (SEQ ID NO:14) or having an at least 50 nucleotide portion of said sequence.

95. An isolated nucleic acid hybridizable under conditions of high stringency to a nucleic acid having the nucleotide sequence depicted in Figure 10 (SEQ ID NO:14) or having an at least 50 nucleotide portion of said sequence.

96. An isolated nucleic acid hybridizable under conditions of high stringency to a nucleic acid having the consensus nucleotide sequence depicted in Figure 13 (SEQ ID NO:38) or having an at least 50 nucleotide portion of said sequence.

97. A purified protein encoded by a first nucleic acid hybridizable to a second nucleic acid having the consensus nucleotide sequence depicted in Figure 13 (SEQ ID NO:38) or having an at least 50 nucleotide portion of said sequence.

98. An isolated nucleic acid comprising a nucleotide sequence that is complementary to the nucleotide sequence of the nucleic acid of claim 92 or 96.

```
      GAATTCGGCACGAGGTTTTTTTTTTTTTTTTCCCCTCTTTTCTTTCTTTTCCTTTTGCC
1     ---------+---------+---------+---------+---------+---------+ 60

      ATCCGAAAGAGCTGTCAGCCGCCGCCGGGCTGCACCTAAAGGCGTCGGTAGGGGGATAAC
61    ---------+---------+---------+---------+---------+---------+ 120

      AGTCAGAGACCCTCCTGAAAGCAGGAGACGGGACGGTACCCCTCCGGCTCTGCGGGGCGG
121   ---------+---------+---------+---------+---------+---------+ 180

      CTGCGGCCCCTCCGTTCTTTCCCCCTCCCCGAGAGACACTCTTCCTTTCCCCCCACGAAG
181   ---------+---------+---------+---------+---------+---------+ 240

      ACACAGGGGCAGGAACGCGAGCGCTGCCCCTCCGCCATGGGAGGCCGCTTCCTGCTGACG
241   ---------+---------+---------+---------+---------+---------+ 300

      CTCGCCCTCCTCTCGGCGCTGCTGTGCCGCTGCCAGGTTGACGGCTCCGGGGTGTTCGAG
301   ---------+---------+---------+---------+---------+---------+ 360

      CTGAAGCTGCAGGAGTTTGTCAACAAGAAGGGGCTGCTCAGCAACCGCAACTGCTGCCGG
361   ---------+---------+---------+---------+---------+---------+ 420

      GGGGGCGGCCCCGGAGGCGCCGGGCAGCAGCAGTGCGACTGCAAGACCTTCTTCCGCGTC
421   ---------+---------+---------+---------+---------+---------+ 480

      TGCCTGAAGCACTACCAGGCCAGCGTCTCCCCCGAGCCGCCCTGCACCTACGGCAGCGCC
481   ---------+---------+---------+---------+---------+---------+ 540

      ATCACCCCCGTCCTCGGCGCCAACTCCTTCAGCGTCCCCGACGGCGCGGGCGGCGCCGAC
541   ---------+---------+---------+---------+---------+---------+ 600

      CCCGCCTTCAGCAACCCCATCCGCTTCCCCTTCGGCTTCACCTGGCCCGGCACCTTCTCG
601   ---------+---------+---------+---------+---------+---------+ 660

      CTCATCATCGAGGCTCTGCACACCGACTCCCCCGACGACCTCACCACAGAAAACCCCGAG
661   ---------+---------+---------+---------+---------+---------+ 720

      CGCCTCATCAGCCGCCTGGCCACCCAGAGGCACCTGGCGGTGGGCGAGGAGTGGTCCCAG
721   ---------+---------+---------+---------+---------+---------+ 780

      GACCTGCACAGCAGCGGCCGCACCGACCTCAAGTACTCCTATCGCTTTGTGTGTGATGAG
781   ---------+---------+---------+---------+---------+---------+ 840
```

FIG. 1A1

```
     CACTACTACGGGGAAGGCTGCTCTGTCTTCTGCCGGCCCCGTGACGACCGCTTCGGTCAC
841  ---------+---------+---------+---------+---------+---------+ 900

     TTCACCTGTGGAGAGCGTGGCGAGAAGGTCTGCAACCCAGGCTGGAAGGGCCAGTACTGC
901  ---------+---------+---------+---------+---------+---------+ 960

     ACTGAGCCGATTTGCTTGCCTGGGTGTGACGAGCAGCACGGCTTCTGCGACAAACCTGGG
961  ---------+---------+---------+---------+---------+---------+ 1020

     GAATGCAAGTGCAGAGTGGGTTGGCAGGGGCGGTACTGTGACGAGTGCATCCGATACCCA
1021 ---------+---------+---------+---------+---------+---------+ 1080

     GGCTGCCTGCACGGTACCTGTCAGCAGCCATGGCAGTGCAACTGCCAGGAAGGCTGGGGC
1081 ---------+---------+---------+---------+---------+---------+ 1140

     GGCCTTTTCTGCAACCAGGACCTGAACTACTGCACTCACCACAAGCCATGCAAGAATGGT
1141 ---------+---------+---------+---------+---------+---------+ 1200

     CGGTGTACGTGGTTGTGGCCAGTCCCCTCGATGTGAACAAGAACGGCTGGACCCATGTGT
1201 ---------+---------+---------+---------+---------+---------+ 1260

     GGCTCCAGCTGCGAGATTGAAATCAACGAATGTGATGCCAACCCTTGCAAGAATGGTGGA
1261 ---------+---------+---------+---------+---------+---------+ 1320

     AGCTGCACGGATCTCGAGAACAGCTATTCCTGTACCTGCCCCCCAGGCTTCTATGGTAAA
1321 ---------+---------+---------+---------+---------+---------+ 1380

     AACTGTGAGCTGAGTGCAATGACTTGTGCTGATGGACCGTGCTTCAATGGAGGGCGATGC
1381 ---------+---------+---------+---------+---------+---------+ 1440

     ACTGACAACCCTGATGGTGGATACAGCTGCCGCTGCCCACTGGGTTATTCTGGGTTCAAC
1441 ---------+---------+---------+---------+---------+---------+ 1500

     TGTGAAAAGAAAATCGATTACTGCAGTTCCAGCCCTTGTGCTAATGGAGCCCAGTGCGTT
1501 ---------+---------+---------+---------+---------+---------+ 1560

     GACCTGGGGAACTCCTACATATGCCAGTGCCAGGCTGGCTTCACTGGCAGGCACTGTGAC
1561 ---------+---------+---------+---------+---------+---------+ 1620

     GACAACGTGGACGATTGCGCCTCCTTCCCCTGCGTCAATGGAGGGACCTGTCAGGATGGG
1621 ---------+---------+---------+---------+---------+---------+ 1680
```

FIG. 1A2

```
     GTCAACGACTACTCCTGCACCTGCCCCCCGGGATACAACGGGAAGAACTGCAGCACGCCG
1681 ---------+---------+---------+---------+---------+---------+ 1740

     GTGAGCAGATGCGAGCACAACCCCTGCCACAATGGGGCCACCTGCCACGAGAGAAGCAAC
1741 ---------+---------+---------+---------+---------+---------+ 1800

     CGCTACGTGTGCGAGTGCGCTCGGGGCTACGGCGGCCTCAACTGCCAGTTCCTGCTCCCC
1801 ---------+---------+---------+---------+---------+---------+ 1860

     GAGCCACCTCAGGGGCCGGTCATCGTTGACTTCACCGAGAAGTACACAGAGGGCCAGAAC
1861 ---------+---------+---------+---------+---------+---------+ 1920

     AGCCAGTTTCCCTGGATCGCAGTGTGCGCCGGGATTATTCTGGTCCTCATGCTGCTGCTG
1921 ---------+---------+---------+---------+---------+---------+ 1980

     TACCAGTCGGTGTACGTCATATCAGAAGAGAAAGATGAGTGCATCATAGCAACTGAGGTG
2401 ---------+---------+---------+---------+---------+---------+ 2460

     TAAAACAGACGTGACGTGGCAAAGCTTATCGATACCGTCATCAAGCTT
2461 ---------+---------+---------+---------+-------- 2508
```

FIG. 1A3

```
   1 GAATTCGGCACGAGGTTTTTTTTTTTTTTTTTCCCCTCTTTTCTTTCTTTTCCTTTTGCCATCCGAAAG   69
  70 AGCTGTCAGCCGCCGCCGGGCTGCACCTAAAGGCGTCGGTAGGGGGATAACAGTCAGAGACCCTCCTGA  138
 139 AAGCAGGAGACGGGACGGTACCCCTCCGGCTCTGCGGGGCGGCTGCGGCCCCTCCGTTCTTTCCCCCTC  207
 208 CCCGAGAGACACTCTTCCTTTCCCCCCACGAAGACACAGGGGCAGGAACGCGAGCGCTGCCCCTCCGCC  276
 277 ATGGGAGGCCGCTTCCTGCTGACGCTCGCCCTCCTCTCGGCGCTGCTGTGCCGCTGCCAGGTTGACGGC  345
 346 TCCGGGGTGTTCGAGCTGAAGCTGCAGGAGTTTGTCAACAAGAAGGGGCTGCTCAGCAACCGCAACTGC  414
 415 TGCCGGGGGGGCGGCCCCGGAGGCGCCGGGCAGCAGCAGTGCGACTGCAAGACCTTCTTCCGCGTCTGC  483
 484 CTGAAGCACTACCAGGCCAGCGTCTCCCCCGAGCCGCCCTGCACCTACGGCAGCGCCATCACCCCCGTC  552
 553 CTCGGCGCCAACTCCTTCAGCGTCCCCGACGGCGCGGGCGGCGCCGACCCCGCCTTCAGCAACCCCATC  621
 622 CGCTTCCCCTTCGGCTTCACCTGGCCCGGCACCTTCTCGCTCATCATCGAGGCTCTGCACACCGACTCC  690
 691 CCCGACGACCTCACCACAGAAAACCCCGAGCGCCTCATCAGCCGCCTGGCCACCCAGAGGCACCTGGCG  759
 760 GTGGGCGAGGAGTGGTCCCAGGACCTGCACAGCAGCGGCCGCACCGACCTCAAGTACTCCTATCGCTTT  828
 829 XXGTGTGATGAGCACTACTACGGGGAAGGCTGCTCTGTCTTCTGCCGGCCCCGTGACGACCGCTTCGGT  897
 898 CACTTCACCTGTGGAGAGCGTGGCGAGAAGGTCTGCAACCCAGGCTGGAAGGGCCAGTACTGCACTGAG  966
 967 CCGATTTGCTTGCCTGGGTGTGACGAGCAGCACGGCTTCTGCGACAAACCTGGGGAATGCAAGTGCAGA 1035
1036 GTGGGTTGGCAGGGGCGGTACTGTGACGAGTGCATCCGATACCCAGGCTGCCTGCACGGTACCTGTCAG 1104
1105 CAGCCATGGCAGTGCAACTGCCAGGAAGGCTGGGGCGGCCTTTTCTGCAACCAGGACCTGAACTACTGC 1173
1174 ACTCACCACAAGCCATGCAAGAATGGTGCCACATGCACCAACACCGGTCAGGGGAGCTACACTTGTTCT 1242
1243 TGCCGACCTGGGTACACAGGCTCCAGCTGCGAGATTGAAATCAACGAATGTGATGCCAACCCTTGCAAG 1311
1312 AATGGTGGAAGCTGCACGGATCTCGAGAACAGCTATTCCTGTACCTGCCCCCCAGGCTTCTATGGTAAA 1380
1381 AACTGTGAGCTGAGTGCAATGACTTGTGCTGATGGACCGTGCTTCAATGGAGGGCGATGCACTGACAAC 1449
1450 CCTGATGGTGGATACAGCTGCCGCTGCCCACTGGGTTATTCTGGGTTCAACTGTGAAAAGAAAATCGAT 1518
1519 TACTGCAGTTCCAGCCCTTGTGCTAATGGAGCCCAGTGCGTTGACCTGGGGAACTCCTACATATGCCAG 1587
1588 TGCCAGGCTGGCTTCACTGGCAGGCACTGTGACGACAACGTGGACGATTGCGCCTCCTTCCCCTGCGTC 1656
1657 AATGGAGGGACCTGTCAGGATGGGGTCAACGACTACTCCTGCACCTGCCCCCCGGGATACAACGGGAAG 1725
1726 AACTGCAGCACGCCGGTGAGCAGATGCGAGCACAACCCCTGCCACAATGGGGCCACCTGCCACGAGAGA 1794
```

FIG. 1B1

1795 AGCAACCGCTACGTGTGCGAGTGCGCTCGGGGCTACGGCGGCCTCAACTGCCAGTTCCTGCTCCCCGAG 1863
1864 CCACCTCAGGGGCCGGTCATCGTTGACTTCACCGAGAAGTACACAGAGGGCCAGAACAGCCAGTTTCCC 1932
1933 TGGATCGCAGTGTGCGCCGGGATTATTCTGGTCCTCATGCTGCTGCTGGGTTGCGCCGCCATCGTCGTC 2001
2002 TGCGTCAGGCTGAAGGTGCAGAAGAGGCACCACCAGCCCGAGGCCTGCAGGAGTGAAACGGAGACCATG 2070
2071 AACAACCTGGCGAACTGCCAGCGCGAGAAGGACATCTCCATCAGCGTCATCGGTGCCACTCAGATTAAA 2139
2140 AACACAAATAAGAAAGTAGACTTTCACAGCGATAACTCCGATAAAAACGGCTACAAAGTTAGATACCCA 2208
2209 TCAGTGGATTACAATTTGGTGCATGAACTCAAGAATGAGGACTCTGTGAAAGAGGAGCATGGCAAATGC 2277
2278 GAAGCCAAGTGTGAAACGTATGATTCAGAGGCAGAAGAGAAAAGCGCAGTACAGCTAAAAAGTAGTGAC 2346
2347 ACTTCTGAAAGAAAACGGCCAGATTCAGTATATTCCACTTCAAAGGACACAAAGTACCAGTCGGTGTAC 2415
2416 GTCATATCAGAAGAGAAAGATGAGTGCATCATAGCAACTGAGGTTAGTATCCCACCTGGCAGTCGGACA 2484
2485 AGTCTTGGTGTGTGATTCCCATCCAGCGCAGGTCAGGGCGGCCAAACCATTCTACCTGCTGCCACAGTC 2553
2554 ATCTGTACCCAATGAAAACTGGCCACCTTCAGTCTGTGGCACTGCAGACGTTGAAAAAACTTGTTGTGG 2622
2623 ATTAACATAAGCTCCAGTGGGGGTTACAGGGACAGCAATTTTTGCAGGCAAGGGTATAACTGTAGTGCA 2691
2692 GTTGTAGCTTACTAACCCTACTGACTCATTCTTTCGTGTGCTTCCTGCAGAGCCTGTTTTTGCTTGGCA 2760
2761 TTGAGGTGAAGTCCTGACCCTCTGCATCCTCATAGTCCTCTGCTTTCTTTTTATTAACCTCTTCTGGTC 2829
2830 TCTGCTTGTGTTTTCTCTCAACAGGTGTAAAACAGACGTGACGTGGCAAAGCTT 2883

FIG. 1B2

```
  1 MGGRFLLTLA LLSALLCRCQ VDGSGVFELK LQEFVNKKGL LSNRNCCRGG GPGGAGQQQC
 61 DCKTFFRVCL KHYQASVSPE PPCTYGSAIT PVLGANSFSV PDGAGGADPA FSNPIRFPFG
121 FTWPGTFSLI IEALHTDSPD DLTTENPERL ISRLATQRHL AVGEEWSQDL HSSGRTDLKY
181 SYRFVCDEHY YGEGCSVFCR PRDDRFGHFT CGERGEKVCN PGWKGQYCTE PICLPGCDEQ
241 HGFCDKPGEC KCRVGWQGRY CDECIRYPGC LHGTCQQPWQ CNCQEGWGGL FCNQDLNYCT
301 HHKPCKNGAT CTNTGQGSYT CSCRPGYTGS SCEIEINECD ANPCKNGGSC TDLENSYSCT
361 CPPGFYGKNC ELSAMTCADG PCFNGGRCTD NPDGGYSCRC PLGYSGFNCE KKIDYCSSSP
421 CANGAQCVDL GNSYICQCQA GFTGRHCDDN VDDCASFPCV NGGTCQDGVN DYSCTCPPGY
481 NGKNCSTPVS RCEHNPCHNG ATCHERSNRY VCECARGYGG LNCQFLLPEP PQGPVIVDFT
541 EKYTEGQNSQ FPWIAVCAGI ILVLMLLLGC AAIVVCVRLK VQKRHHQPEA CRSETETMNN
601 LANCQREKDI SISVIGATQI KNTNKKVDFH SDNSDKNGYK VRYPSVDYNL VHELKNEDSV
661 KEEHGKCEAK CETYDSEAEE KSAVQLKSSD TSERKRPDSV YSTSKDTKYQ SVYVISEEKD
721 ECIIATEV
```

FIG. 2

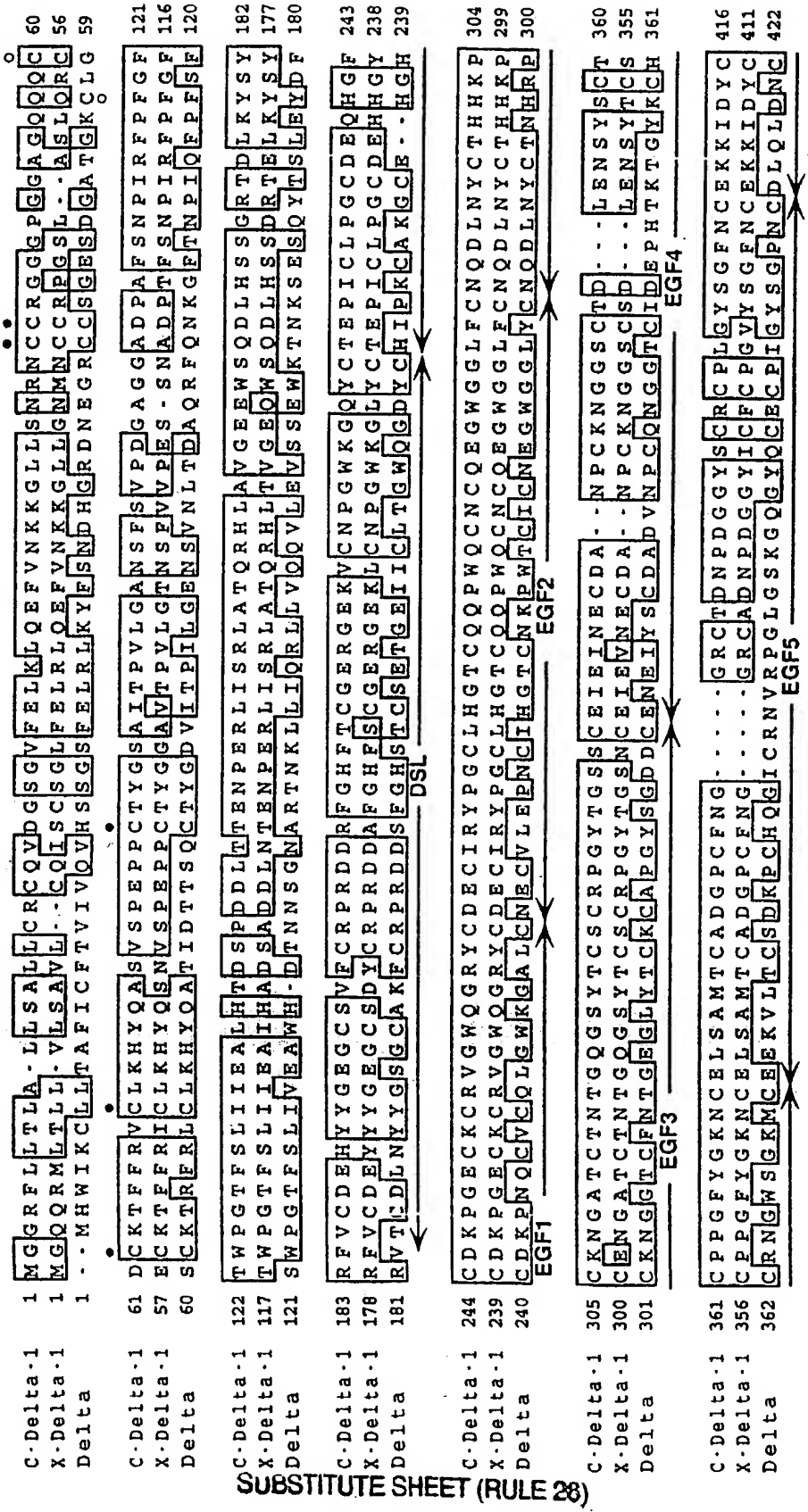

FIG. 3A

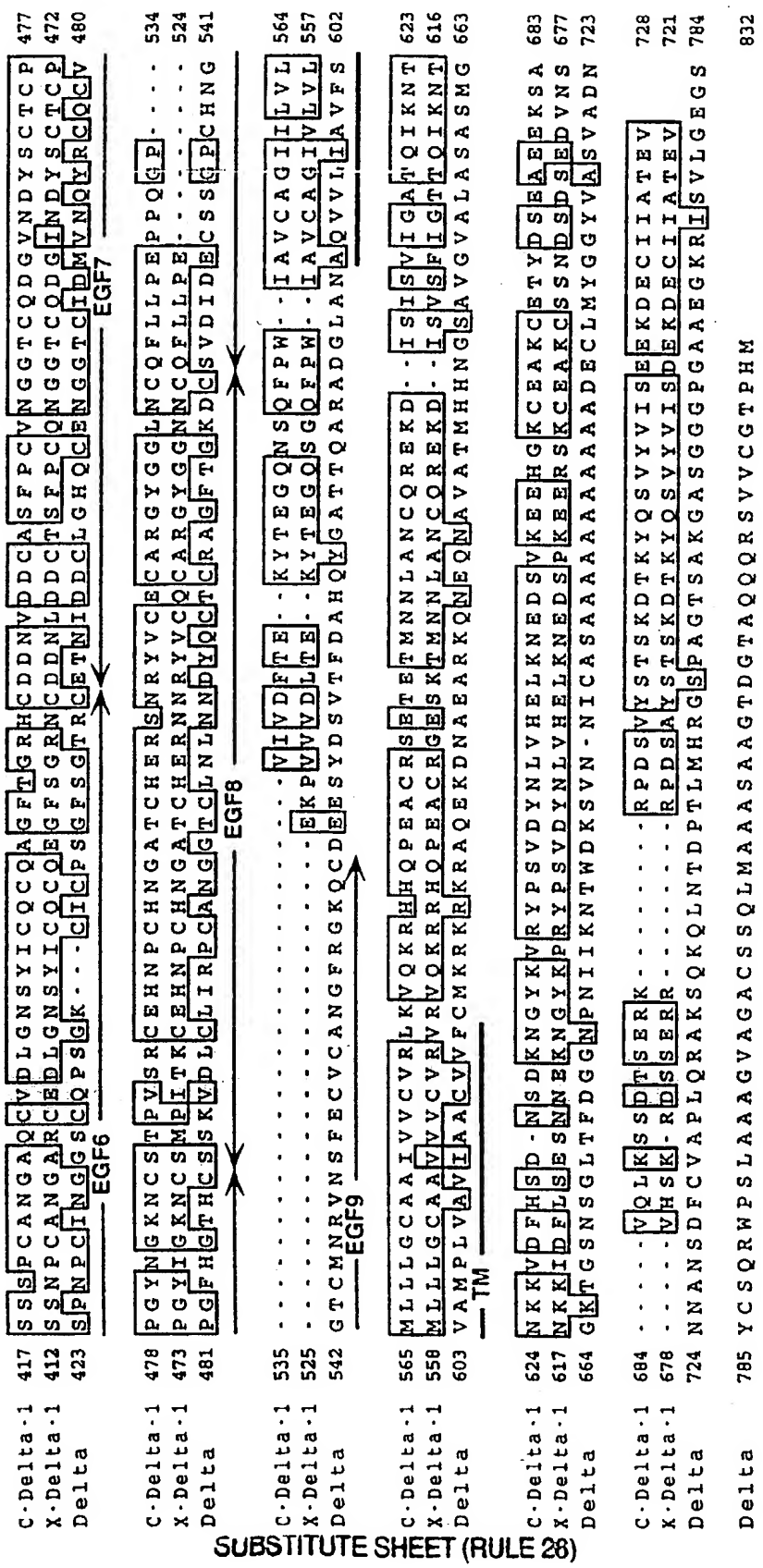

FIG. 3B

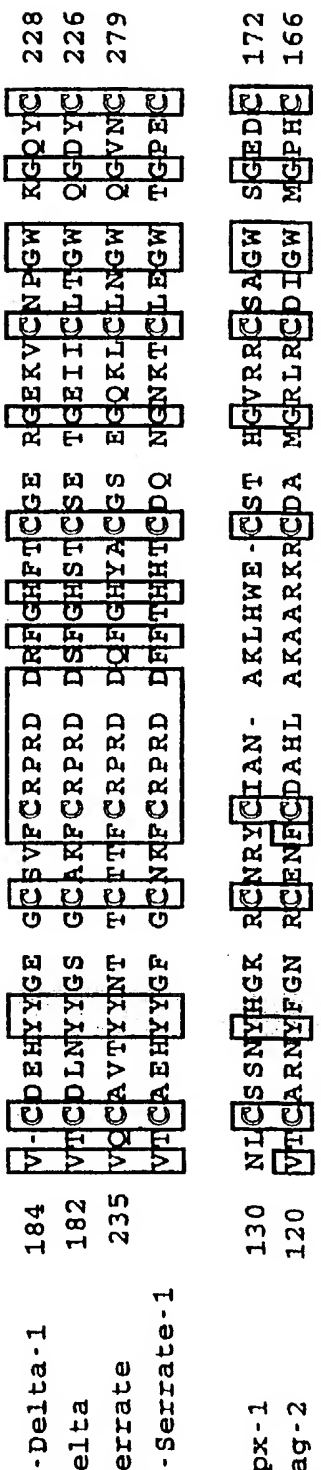
C-Delta-1 184 V-CDEHYYGE GCSVFCRPRD DRFGHFTCGE RGEKVCNPGW KGQYC 228
Delta 182 VTCDLNYYGS GCAKFCRPRD DSFGHSTCSE TGEIICLTGW QGDYC 226
Serrate 235 VQCAVTYYNT TCTTFCRPRD DQFGHYACGS EGQKLCLNGW QGVNC 279
C-Serrate-1 VTCAEHYYGF GCNKFCRPRD DFFTHHTCDQ NGNKTCLEGW TGPEC
Apx-1 130 NLCSSNYHGK RCNRYCIAN- AKLHWE-CST HGVRRCSAGW SGEDC 172
Lag-2 120 VTCARNYFGN RCENFCDAHL AKAARKRCDA MGRLRCDIGW MGPHC 166

FIG. 4

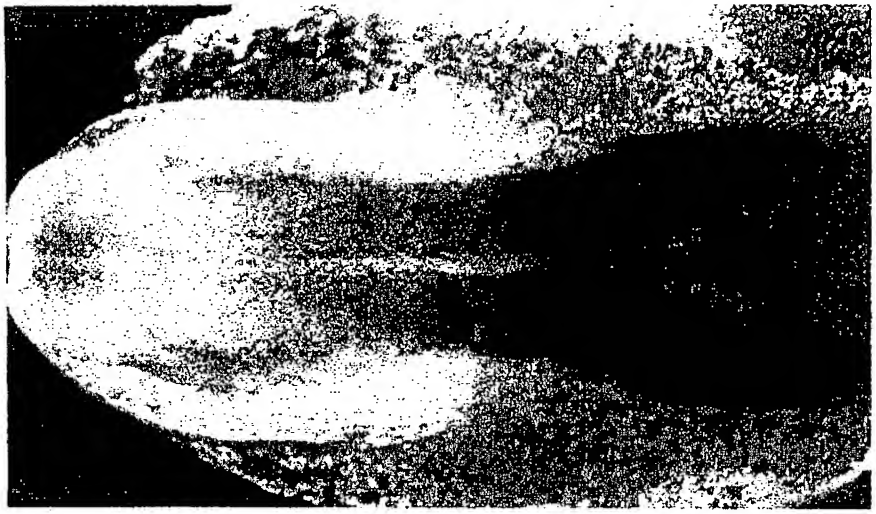

FIG.5A

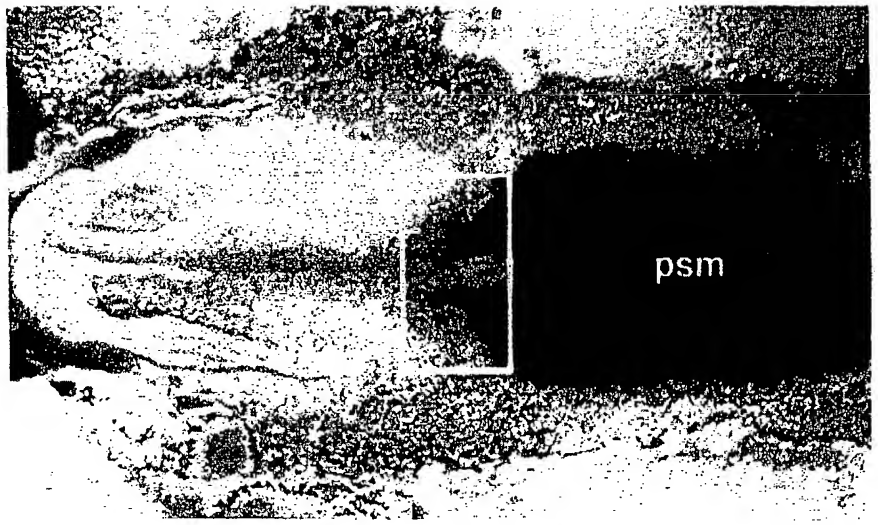

FIG.5B

FIG.5C

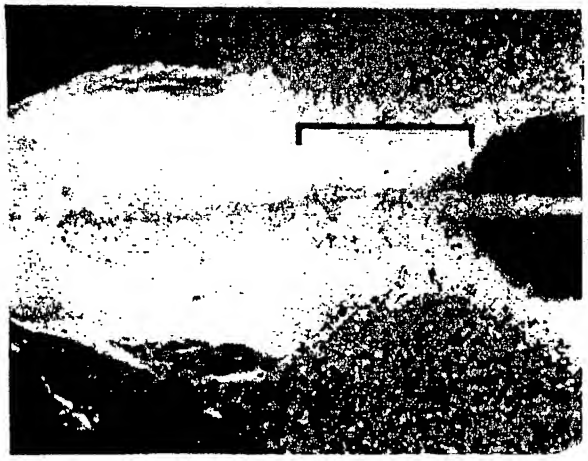

FIG.5D

FIG.5E

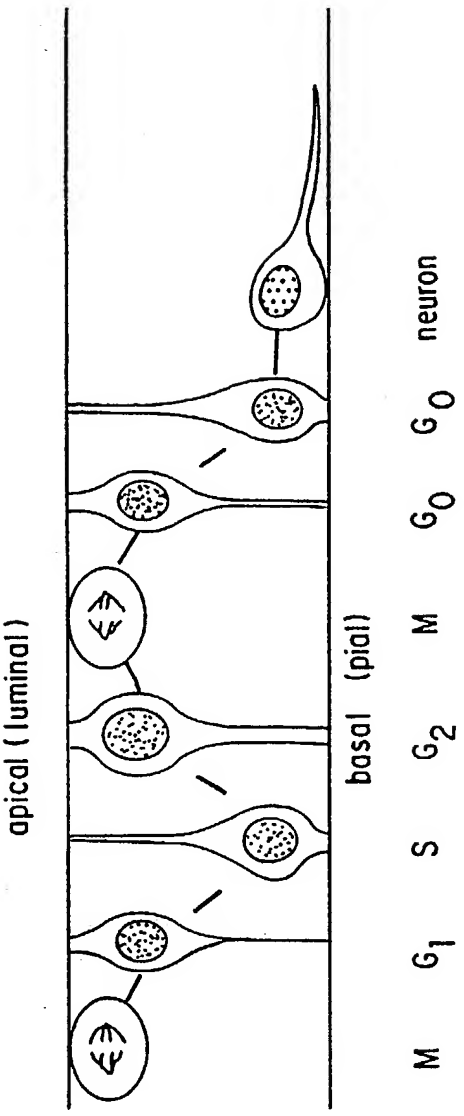

FIG. 6A

FIG.6B

FIG.6C

```
CTGCAGGAAT TCSMYCGCAT GCTCCCGGCC GCCATGGGCC GTCGGAGCGC GCTAGCCCTT      60
GCCGTGGTCT CTGCCCTGCT GTGCCAGGTC TGGAGCTCCG GCGTATTTGA GCTGAAGCTG     120
CAGGAGTTCG TCAACAAGAA GGGGCTGCTG GGGAACCGCA ACTGCTGCCG CGGGGGCTCT     180
GGCCCGCCTT GCGCCTGCAG GACCTTCTTT CGCGTATGCC TCAAGCACTA CCAGGCCAGC     240
GTGTCACCGG AGCCACCCTG CACCTACGGC AGTGCCGTCA CGCCAGTGCT GGGTGTCGAC     300
TCCTTCAGCC TGCCTGATGG CGCAGGCATC GACCCCGCCT TCAGCAACCC CATCCGATTC     360
CCCTTCGGCT TCACCTGGCC AGGTACCTTC TCTCTGATCA TTGAAGCCCT CCATACAGAC     420
TCTCCCGATG ACCTCGCAAC AGAAAACCCA GAAAGACTCA TCAGCCGCCT GACCACACAG     480
AGGCACCTCA CTGTGGGAGA AGAATGGTCT CAGGACCTTC ACAGTAGCGG CCGCACAGAC     540
CTCCGGTACT CTTACCGGTT TGTGTGTGAC GAGCACTACT ACGGAGAAGG TTGCTCTGTG     600
TTCTGCCGAC CTCGGGATGA CGCCTTTGGC CACTTCACCT GCGGGGACAG AGGGGAGAAG     660
ATGTGCGACC CTGGCTGGAA AGGCCAGTAC TGCACTGACC CAATCTGTCT GCCAGGGTGT     720
GATGACCAAC ATGGATACTG TGACAAACCA GGGGAGTGCA AGTGCAGAGT TGGCTGGCAG     780
GGCCGCTACT GCGATGAGTG CATCCGATAC CCAGGTTGTC TCCATGGCAC CTGCCAGCAA     840
CCCTGGCAGT GTAACTGCCA GGAAGGCTGG GGGGGCCTTT TCTGCAACCA AGACCTGAAC     900
TACTGTACTC ACCATAAGCC GTGCAGGAAT GGAGCCACCT GCACCAACAC GGGCCAGGGG     960
AGCTACACAT GTTCCTGCCG ACCTGGGTAT ACAGGTGCCA ACTGTGAGCT GGAAGTAGAT    1020
GAGTGTGCTC CTAGCCCCTG CAAGAACGGA GCGAGCTGCA CGGACCTTGA GGACAGCTTC    1080
TCTTGCACCT GCCCTCCCGG CTTCTATGGC AAGGTCTGTG AGCTGAGCGC CATGACCTGT    1140
GCAGATGGCC CTTGCTTCAA TGGAGGACGA TGTTCAGATA ACCCTGACGG AGGCTACACC    1200
TGCCATTGCC CCTTGGGCTT CTCTGGCTTC AACTGTGAGA AGAAGATGGA TCTCTGCGGC    1260
TCTTCCCCTT GTTCTAACGG TGCCAAGTGT GTGGACCTCG GCAACTCTTA CCTGTGCCGG    1320
TGCCAGGCTG GCTTCTCCGG GAGGTACTGC GAGGACAATG TGGATGACTG TGCCTCCTCC    1380
```

FIG. 7A

```
CCGTGTGCAA ATGGGGGCAC CTGCCGGGAC AGTGTGAACG ACTTCTCCTG TACCTGCCCA    1440
CCTGGCTACA CGGGCAAGAA CTGCAGCGCC CCTGTCAGCA GGTGTGAGCA TGCACCCTGC    1500
CATAATGGGG CCACCTGCCA CCAGAGGGGC CAGCGCTACA TGTGTGAGTG CGCCCAGGGC    1560
TATGGCGGCC CCAACTGCCA GTTTCTGCTC CCTGAGCCAC CACCAGGGCC CATGGTGGTG    1620
GACCTCAGTG AGAGGCATAT GGAGAGCCAG GGCGGGCCCT TCCCCTGGGT GGCCGTGTGT    1680
GCCGGGGTGG TGCTTGTCCT CCTGCTGCTG CTGGGCTGTG CTGCTGTGGT GGTCTGCGTC    1740
CGGCTGAAGC TACAGAAACA CCAGCCTCCA CCTGAACCCT GTGGGGGAGA GACAGAAACC    1800
ATGAACAACC TAGCCAATTG CCAGCGCGAG AAGGACGTTT CTGTTAGCAT CATTGGGGCT    1860
ACCCAGATCA AGAACACCAA CAAGAAGGCG GACTTTCACG GGGACCATGG AGCCGAGAAG    1920
AGCAGCTTTA AGGTCCGATA CCCCACTGTG GACTATAACC TCGTTCGAGA CCTCAAGGGA    1980
GATGAAGCCA CGGTCAGGGA TACACACAGC AAACGTGACA CCAAGTGCCA GTCACAGAGC    2040
TCTGCAGGAG AAGAGAAGAT CGCCCCAACA CTTAGGGGTG GGGAGATTCC TGACAGAAAA    2100
AGGCCAGAGT CTGTCTACTC TACTTCAAAG GACACCAAGT ACCAGTCGGT GTATGTTCTG    2160
TCTGCAGAAA AGGATGAGTG TGTTATAGCG ACTGAGGTGT AAGATGGAAG CGATGTGGCA    2220
AAATTCCCAT TTCTCTTAAA TAAAATTCCA AGGATATAGC CCCGATGAAT GCTGCTGAGA    2280
GAGGAAGGGA GAGGAAACCC AGGGACTGCT GCTGAGAACC AGGTTCAGGC GAACGTGGTT    2340
CTCTCAGAGT TAGCAGAGGC GCCCGACACT GCCAGCCTAG GCTTTGGCTG CCGCTGGACT    2400
GCCTGCTGGT TGTTCCCATT GCACTATGGA CAGTTGCTTT GAAGAGTATA TATTTAAATG    2460
GACGAGTGAC TTGATTCATA TAGGAAGCAC GCACTGCCCA CACGTCTATC TTGGATTACT    2520
ATGAGCCAGT CTTTCCTTGA ACTAGAAACA CAACTGCCTT TATTGTCCTT TTTGATACTG    2580
AGATGTGTTT TTTTTTTTTC CTAGACGGGA AAAAGAAAAC GTGTGTTATT TTTTTTGGGA    2640
TTTGTAAAAA TATTTTTCAT GATTATGGGA GAGCTCCCAA CGCGTTGGAG GT            2692
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FIG. 7B

```
MGRRSALALA VVSALLCQVW SSGVFELKLQ EFVNKKGLLG NRNCCRGGSG      50
PPCACRTFFR VCLKHYQASV SPEPPCTYGS AVTPVLGVDS FSLPDGAGID     100
PAFSNPIRFP FGFTWPGTFS LIIEALHTDS PDDLATENPE RLISRLTTQR     150
HLTVGEEWSQ DLHSSGRTDL RYSYRFVCDE HYYGEGCSVF CRPRDDAFGH     200
FTCGDRGEKM CDPGWKGQYC TDPICLPGCD DQHGYCDKPG ECKCRVGWQG     250
RYCDECIRYP GCLHGTCQQP WQCNCQEGWG GLFCNQDLNY CTHHKPCRNG     300
ATCTNTGQGS YTCSCRPGYT GANCELEVDE CAPSPCKNGA SCTDLEDSFS     350
CTCPPGFYGK VCELSAMTCA DGPCFNGGRC SDNPDGGYTC HCPLGFSGFN     400
CEKKMDLCGS SPCSNGAKCV DLGNSYLCRC QAGFSGRYCE DNVDDCASSP     450
CANGGTCRDS VNDFSCTCPP GYTGKNCSAP VSRCEHAPCH NGATCHQRGQ     500
RYMCECAQGY GGPNCQFLLP EPPPGPMVVD LSERHMESQG GPFPWVAVCA     550
GVVLVLLLLL GCAAVVVCVR LKLQKHQPPP EPCGGETETM NNLANCQREK     600
DVSVSIIGAT QIKNTNKKAD FHGDHGAEKS SFKVRYPTVD YNLVRDLKGD     650
EATVRDTHSK RDTKCQSQSS AGEEKIAPTL RGGEIPDRKR PESVYSTSKD     700
TKYQSVYVLS AEKDECVIAT EV                                   722
```

FIG. 8

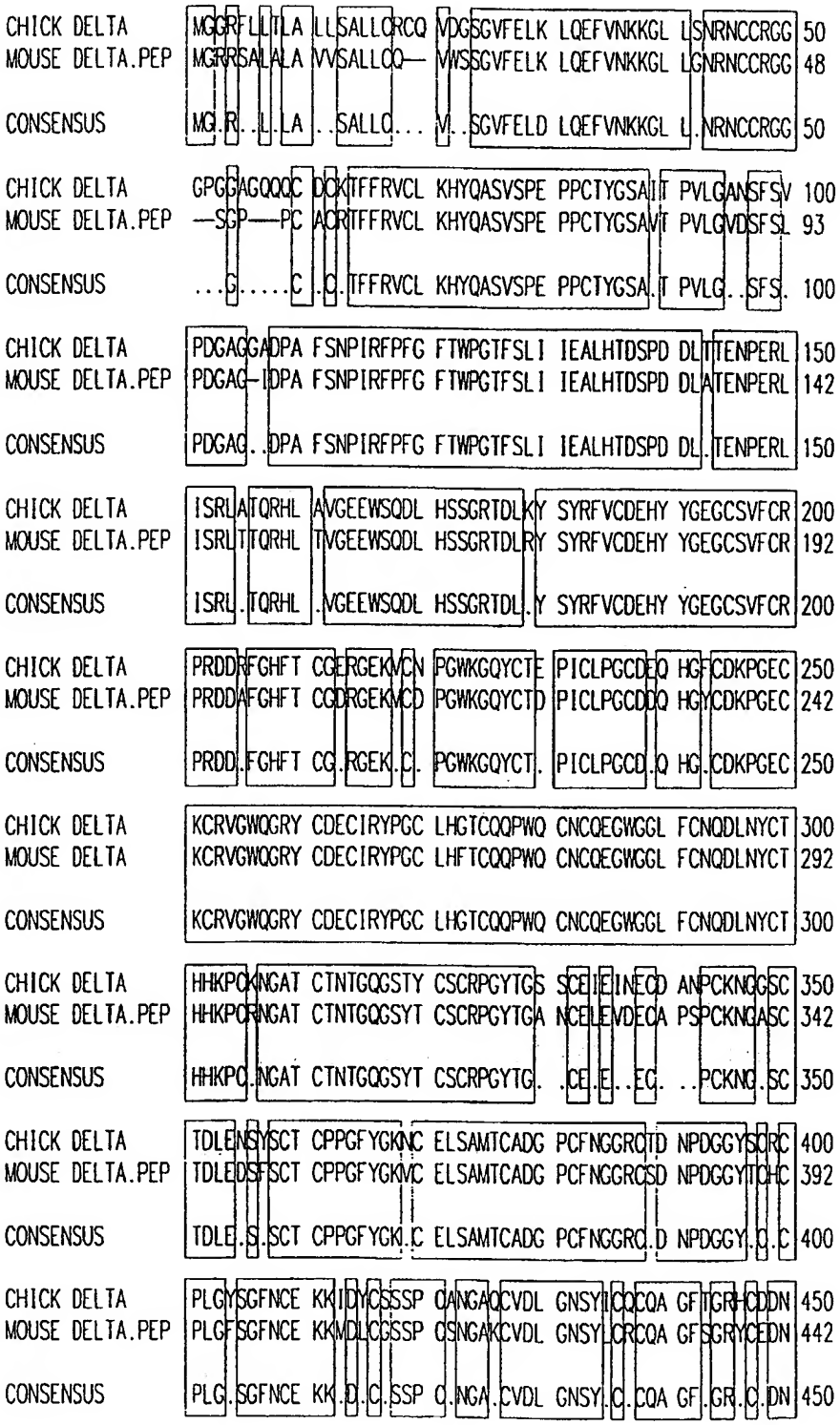


FIG.9A

SUBSTITUTE SHEET (RULE 26)

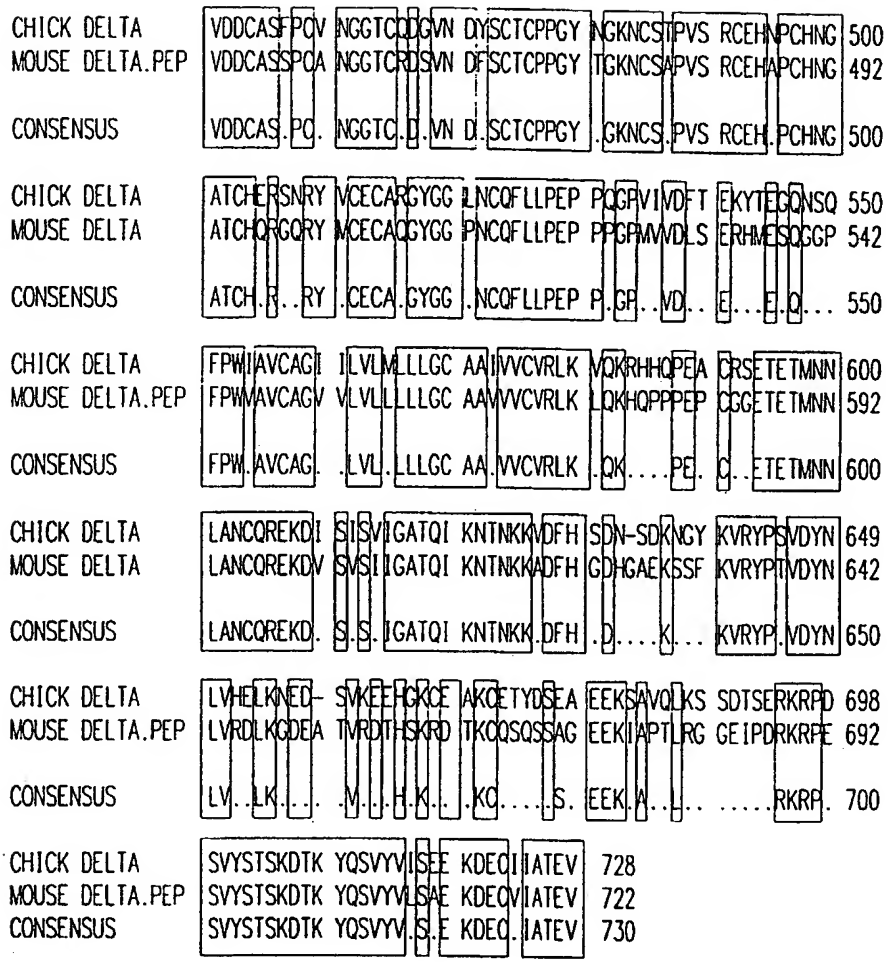

CHICK DELTA VDDCASFPCV NGGTCQDGVN DYSCTCPPGY NGKNCSTPVS RCEHNPCHNG 500
MOUSE DELTA.PEP VDDCASSPCA NGGTCRDSVN DFSCTCPPGY TGKNCSAPVS RCEHAPCHNG 492
CONSENSUS VDDCAS.PC. NGGTC.D.VN D.SCTCPPGY .GKNCS.PVS RCEH.PCHNG 500
CHICK DELTA ATCHERSNRY VCECARGYGG PNCQFLLPEP PQGPVIVDFT EKYTEGQNSQ 550
MOUSE DELTA ATCHQRGQRY MCECAQGYGG PNCQFLLPEP PPGPMVVDLS ERHMESQGGP 542
CONSENSUS ATCH.R..RY .CECA.GYGG .NCQFLLPEP P.GP..VD.. E...E.Q... 550
CHICK DELTA FPWIAVCAGI ILVLMLLLGC AAIVVCVRLK VQKRHHQPEA CRSETETMNN 600
MOUSE DELTA.PEP FPWMAVCAGV VLVLLLLLGC AAVVVCVRLK LQKHQPPPEP CGGETETMNN 592
CONSENSUS FPW.AVCAG. .LVL.LLLGC AA.VVCVRLK .QK....PE. C..ETETMNN 600
CHICK DELTA LANCQREKDI SISVIGATQI KNTNKKVDFH SDN-SDKNGY KVRYPSVDYN 649
MOUSE DELTA LANCQREKDV SVSIIGATQI KNTNKKADFH GDHGAEKSSF KVRYPTVDYN 642
CONSENSUS LANCQREKD. S.S.IGATQI KNTNKK.DFH .D....K... KVRYP.VDYN 650
CHICK DELTA LVHELKNED- SVKEEHGKCE AKCETYDSEA EEKSAVQLKS SDTSERKRPD 698
MOUSE DELTA.PEP LVRDLKGDEA TVRDTHSKRD TKCQSQSSAG EEKIAPTLRG GEIPDRKRPE 692
CONSENSUS LV..LK.... .V...H.K.. .KC....S. EEK.A..L.. .....RKRP. 700
CHICK DELTA SVYSTSKDTK YQSVYVISEE KDECIIATEV 728
MOUSE DELTA.PEP SVYSTSKDTK YQSVYVLSAE KDECVIATEV 722
CONSENSUS SVYSTSKDTK YQSVYV.S.E KDEC.IATEV 730

FIG.9B

```
      10         20         30         40         50         60
                 *                     *                     *
TACGATGAAY AACCTGGCGA ACTGCCAGCG TCAGAAGGAC ATCTCAGTCA GCATCATCGG
 Y  D  E  X  P  G  E  L  P  A  *  E  G  H  L  S  Q  H  H  R>
  T  M  N  N  L  A  N  C  Q  R  E  K  D  I  S  V  S  I  I  G>
   R  *  X  T  W  R  T  A  S  V  R  R  T  S  Q  S  A  S  S>

      70         80         90        100        110        120
                 *                     *                     *
GGCYACGTCA GATCARGAAC ACCAACAAGA AGGCGGACTT YMCASCGGGG GACCASAGCG
 G  X  V  R  S  X  T  P  T  R  R  R  T  X  X  R  G  T  X  A>
  A  T  S  D  Q  E  H  Q  Q  E  G  G  L  X  X  G  G  P  X  R>
G  X  R  Q  I  X  N  T  N  K  K  A  D  F  X  X  G  D  X  S>

     130        140        150        160        170        180
                 *                     *                     *
TCCGACAAGA ATGGMTTTCA AGGCCYGCTA CCCCAGCGTG GACTATAACT CGTGCAGGAC
 S  D  K  N  G  F  Q  G  P  L  P  Q  R  G  L  *  L  V  Q  D>
  P  T  R  M  X  F  K  A  R  Y  P  S  V  D  Y  N  S  C  R  T>
V  R  Q  E  W  X  S  R  P  A  T  P  A  W  T  I  T  R  A  G>

     190        200        210        220        230        240
                 *                     *                     *
CTCAAGGGTG ACGACACCGC CGTCAGGACG TCGCACAGCA AGCGTGACAC CAAGTGCCAG
 L  K  G  D  D  T  A  V  R  T  S  H  S  K  R  D  T  K  C  Q>
  S  R  V  T  T  P  P  S  G  R  R  T  A  S  V  T  P  S  A  S>
P  Q  G  *  R  H  R  R  Q  D  V  A  Q  Q  A  *  H  Q  V  P>

     250        260        270        280        290        300
                 *                     *                     *
TCCCCAGGCT CCTCAGGGAG GAGAAGGGGA CCCCGACCAC ACTCAGGGGK TGCGTGCTGC
 S  P  G  S  S  G  R  R  R  G  P  R  P  H  S  G  X  A  C  C>
  P  Q  A  P  Q  G  G  E  G  D  P  D  H  T  Q  G  X  R  A  A>
V  P  R  L  L  R  E  E  K  G  T  P  T  T  L  R  G  C  V  L>

     310        320        330        340        350        360
                 *                     *                     *
GGGCCGGGCT CAGGAGGGGG TACCTGGGGG GTGTCTTCCT GGAACCACTG CTCCGTTTCT
 G  P  G  S  G  G  G  T  W  G  V  S  S  W  N  H  C  S  V  S>
  G  R  A  Q  E  G  V  P  G  G  C  L  P  G  T  T  A  P  F  L>
R  A  G  L  R  R  G  Y  L  G  G  V  F  L  E  P  L  L  R  F>
```

FIG. 10A

```
       370        380        390        400        410        420
                   *                     *                     *
CTTCCCAAAT GTTCTCATGC ATTCATTGTG GATTTTCTCT ATTTTCCTTT TAGTGGAGAA
 L  P  K   C  S  H  A   F  I  V   D  F  L   Y  F  P  F   S  G  E>
  F  P  N   V  L  M   H  S  L  W   I  F  S   I  F  L   L  V  E  K>
S  S  Q  M  F  S  C   I  H  C   G  F  S  L   F  S  F   *  W  R>

       430        440        450        460        470        480
                   *                     *                     *
GCATCTGAAA GAAAAAGGCC GGACTCGGGC TGTTCAACTT CAAAAGACAC CAAGTACCAG
 A  S  E   R  K  R  P   D  S  G   C  S  T   S  K  D  T   K  Y  Q>
  H  L  K   E  K  G   R  T  R  A   V  Q  L   Q  K  T   P  S  T  S>
S  I  *  K   K  K  A   G  L  G  L   F  N  F   K  R  H   Q  V  P>

       490        500        510        520
                   *                     *
TCGGTGTACG TCATATCCGA GGAGAAGGAC GAGTGCGTCA TCGCA
 S  V  Y   V  I  S  E   E  K  D   E  C  V   I   A>
  R  C  T   S  Y  P   R  R  R  T   S  A  S   S>
V  G  V  R   H  I  R   G  E  G   R  V  R  H   R>
```

FIG. 10B

```
  1 TMNNLANCQREKDISVSIIGATQIXNTNKKADFXXGDXSSDKNGFQKARY  50
    |||||||||||||||:|:||||||||||||.|| ::   |||||: |:.||
597 TMNNLANCQREKDISISVIGATQIKNTNKKVDFHSDN..SDKNGY.KVRY 643

 51 PSVDYNLVQDLKGDDTAVRTSHSKRDTKCQSPGSSGRRRGPRPHSGXACC 100
    ||||||||::||.:| .|:..|:| :.||: .:|.:  :::
644 PSVDYNLVHELKNED.SVKEEHGKCEAKCETYDSEAEEKSA........  683

101 GPGSGGGTWGVSSWNHCSVSLPKCSHAFIVDFLYFPFSGEASERKRPDSG 150
                                |::    |::.||||||||.
684 ............................VQLK....SSDTSERKRPDSV 700

151 CSTSKDTKYQSVYVISEEKDECVIA  175
    :|||||||||||||||||||:||
701 YSTSKDTKYQSVYVISEEKDECIIA  725
```

FIG.11

```
      10         20         30         40         50         60
   *   *   *   *   *   *   *   *   *   *   *   *
CATTGGGTAC GGGCCCCCCT CGAGGTCGAC GGTATCGATA AGCTTGATAT CGAATTCCGG
      70         80         90        100        110        120
   *   *   *   *   *   *   *   *   *   *   *   *
CTTCACCTGG CCGGGCACCT TCTCTCTGAT TATTGAAGCT CTCCACACAG ATTCTCCTGA
     130        140        150        160        170        180
   *   *   *   *   *   *   *   *   *   *   *   *
TGACCTCGCA ACAGAAAACC CAGAAAGACT CATCAGCCGC CTGGCCACCC AGAGGCACCT
     190        200        210        220        230        240
   *   *   *   *   *   *   *   *   *   *   *   *
GACGGTGGGC GAGGAGTGGT CCCAGGACCT GCACAGCAGC GGCCGCACGG ACCTCAAGTA
     250        260        270        280        290        300
   *   *   *   *   *   *   *   *   *   *   *   *
CTCCTACCGC TTCGTGTGTC ACCAACACTA CTACGGAGAG GGCTGCTCCG TTTTCTGCCG
     310        320        330        340        350        360
   *   *   *   *   *   *   *   *   *   *   *   *
TCCCCGGGAC GATGCCTTCG GCCACTTCAC CTGTGGGGAG CGTGGGGAGA AAGTGTGCAA
     370        380        390        400        410        420
   *   *   *   *   *   *   *   *   *   *   *   *
CCCTGGCTCG AAAGGGCCCT ACTGCACAGA GCCGATCTGC CTGCCTGGAT GTGATGAGCA
     430        440        450        460        470        480
   *   *   *   *   *   *   *   *   *   *   *   *
GCATGGATTT TGTGACAAAC CAGGGGAATG CAAGTGCAGA GTGGGCTGGC AGGGCCGGTA
     490        500        510        520        530        540
   *   *   *   *   *   *   *   *   *   *   *   *
GTGTGACGAG TGTATCCGCT ATCCAGGCTG TCTCCATGGC ACCTGCCAGC AGCCCTGGCA
     550        560        570        580        590        600
   *   *   *   *   *   *   *   *   *   *   *   *
GTGCAACTGC CAGGAAGGNT GGGGGGGCCT TTTCTGCAAC CAGGACCTGA ACTACTGCAC
     610        620        630        640        650        660
   *   *   *   *   *   *   *   *   *   *   *   *
ACACCATAAG CCCTGCAAGA ATGGAGCCAC CTGCAACAAA CACGGGCCAG GGGGAGCTAC
     670        680        690        700        710        720
   *   *   *   *   *   *   *   *   *   *   *   *
ACTTGGTCTT TGGCCGGNCT GGGGTACANA GGGTGCCACC TGCGAAGCTT GGGGATTGGA
     730        740        750        760        770        780
   *   *   *   *   *   *   *   *   *   *   *   *
CGAGTTGTTG ACCCCAGCCC TTGGTAAGAA CGGAGGGAGC TTGACGGATC TTCGGAGAAC
     790        800        810        820        830        840
   *   *   *   *   *   *   *   *   *   *   *   *
AGCTACTCCT GTACCTGCCC ACCCGGCTTC TACGGCAAAA TCTGTGAATT GAGTGCCATG
     850        860        870        880        890        900
   *   *   *   *   *   *   *   *   *   *   *   *
ACCTGTGCGG ACGGCCCTTG CTTTAACGGG GGTCGGTGCT CAGACAGCCC CGATGGAGGG
```

FIG. 12A1

```
      910         920         930         940         950         960
   *   *   *   *   *   *   *   *   *   *   *   *
TACAGCTGCC GCTGCCCCGT GGGCTACTCC GGCTTCAACT GTGAGAAGAA AATTGACTAC
      970         980         990        1000        1010        1020
   *   *   *   *   *   *   *   *   *   *   *   *
TGCAGCTCTT CACCCTGTTC TAATGGTGCC AAGTGTGTGG ACCTCGGTGA TGCCTACCTG
     1030        1040        1050        1060        1070        1080
   *   *   *   *   *   *   *   *   *   *   *   *
TGCCGCTGCC AGGCCGGCTT CTCGGGGAGG CACTGTGACG ACAACGTGGA CGACTGCGCC
     1090        1100        1110        1120        1130        1140
   *   *   *   *   *   *   *   *   *   *   *   *
TCCTCCCCGT GCGCCAACGG ACCTCGGTGA CGGGATGGCG TGAACGACTT CTCCTGCACC
     1150        1160        1170        1180        1190        1200
   *   *   *   *   *   *   *   *   *   *   *   *
TGCCCGCCTG GCTACACGGG CAGGAACTGC AGTGCCCCCG CCAGCACCTG CGAGCACGCA
     1210        1220        1230        1240        1250        1260
   *   *   *   *   *   *   *   *   *   *   *   *
CCCTGCCACA ATGGGGCCAC CTGCCACGAG AGGGGCCACC GCTATNTGTG CGAGCACGCA
     1270        1280        1290        1300        1310        1320
   *   *   *   *   *   *   *   *   *   *   *   *
CGAAGCTACG GGGGTCCCAA CTCCCANTTC CTGCTCCCCC AAACTGCCCC CCCGGCCCCA
     1330        1340        1350        1360        1370        1380
   *   *   *   *   *   *   *   *   *   *   *   *
CGGTGGTGGA AACTCCCCTA AAAAAACCTA AAAGGGCCGG GGGGGGCCCA TCCCCTTGGT
     1390        1400        1410        1420        1430        1440
   *   *   *   *   *   *   *   *   *   *   *   *
GGACGTGTGC GCCGGGGTCA TCCTTGTCCT CATGCTGCTG CTGGGCTGTG CCGCTGTGGT
     1450        1460        1470        1480        1490        1500
   *   *   *   *   *   *   *   *   *   *   *   *
GGTCTGCGTC CGGCTGAGGC TGCAGAAGCA CCGGCCCCCA GCCGACCCCT GNCGGGGGGA
     1510        1520        1530        1540        1550        1560
   *   *   *   *   *   *   *   *   *   *   *   *
GACGGAGACC ATGAACAACC TGGNCAACTG CCAGCGTGAG AAGGACATCT CAGTCAGCAT
     1570        1580        1590        1600        1610        1620
   *   *   *   *   *   *   *   *   *   *   *   *
CATCGGGGNC ACGCAGATCA AGAACACCAA CAAGAAGGCG GACTTCCACG GGGACCACAG
     1630        1640        1650        1660        1670        1680
   *   *   *   *   *   *   *   *   *   *   *   *
NGCCGACAAG AATGGCTTCA AGGCCCGCTA CCCAGNGGTG GACTATAACC TCGTGCAGGA
     1690        1700        1710        1720        1730        1740
   *   *   *   *   *   *   *   *   *   *   *   *
CCTCAAGGGT GACGACACCG CCGTCAGCCA CGCGCACAGC AAGCGTGACA CCAAGTGNCA
     1750        1760        1770        1780        1790        1800
   *   *   *   *   *   *   *   *   *   *   *   *
GCCCCAGGGC TCCTCAGGGG AGGAGAAGGG GACCCCCGAC CCACACTCAG GGGGTGGAGG
```

FIG.12A2

```
     1810       1820       1830       1840       1850       1860
   *   *    *   *    *   *    *   *    *   *    *   *
AAGCATCTTG AAAGAAAAAG GCCGGACTTC GGGCTTGTTC AACTTTCAAA AGACAANCAA
     1870       1880       1890       1900       1910       1920
   *   *    *   *    *   *    *   *    *   *    *   *
NGTACAAGTC GGTGTNCGTC ATTTCCGNAG GAGGAAGGNT GACTGCGTCA TAGGAANTTG
     1930       1940       1950       1960       1970       1980
   *   *    *   *    *   *    *   *    *   *    *   *
AGGTNGTAAA NTGGNAGTTG ANNTTGGAAA GNNNTCCCCG GATTCCGNTT TCAAAGTTTT

T
```

FIG. 12A3

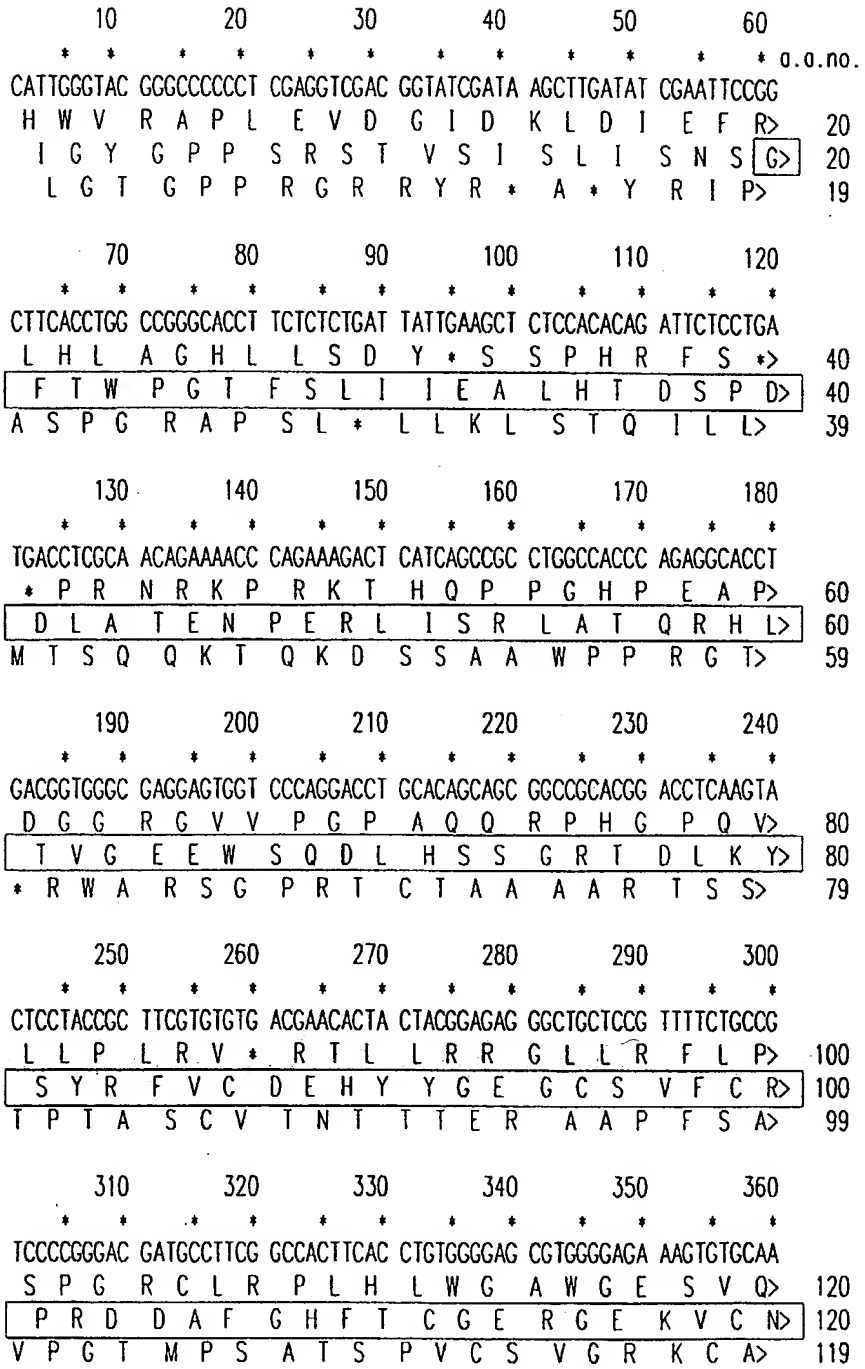
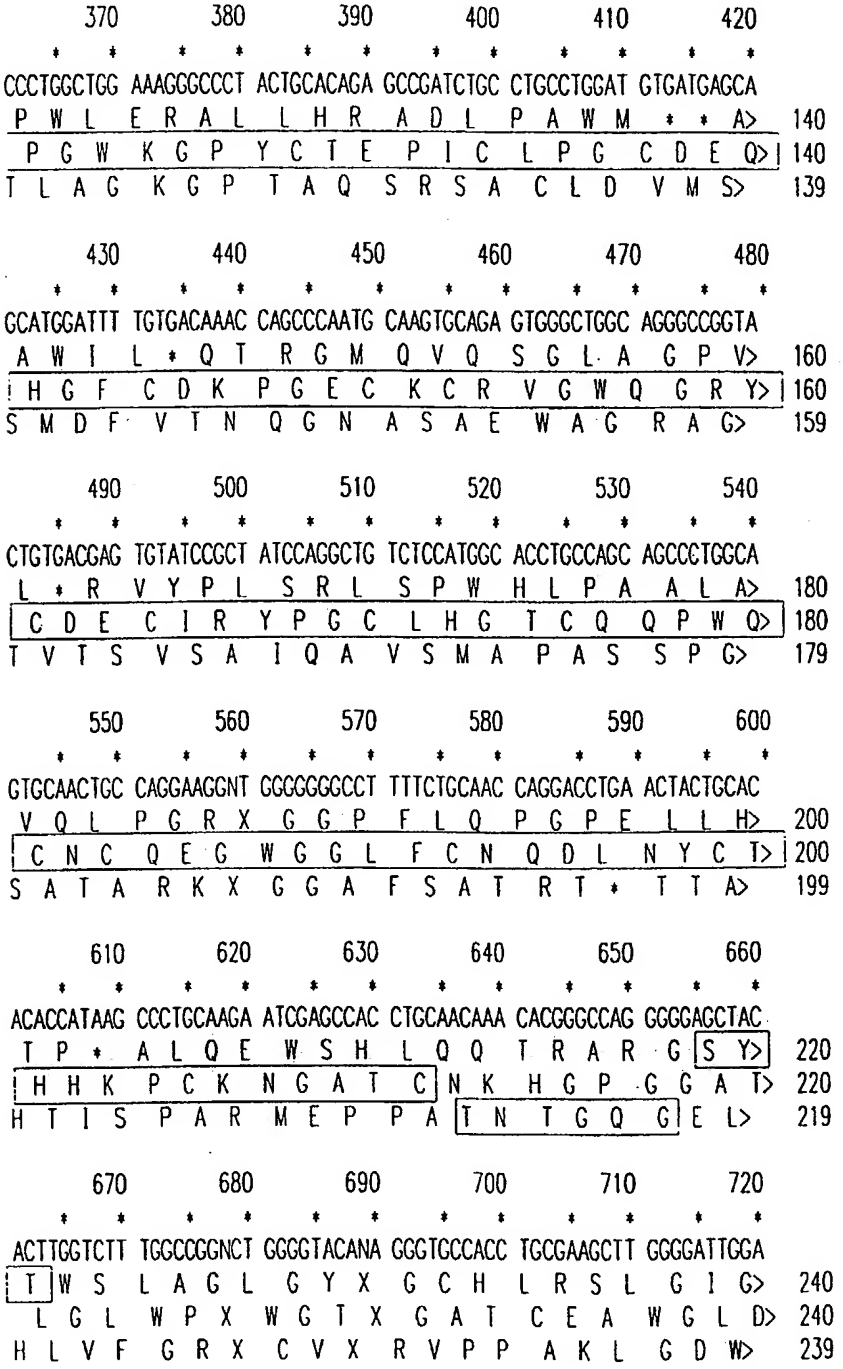


FIG.12B1

SUBSTITUTE SHEET (RULE 26)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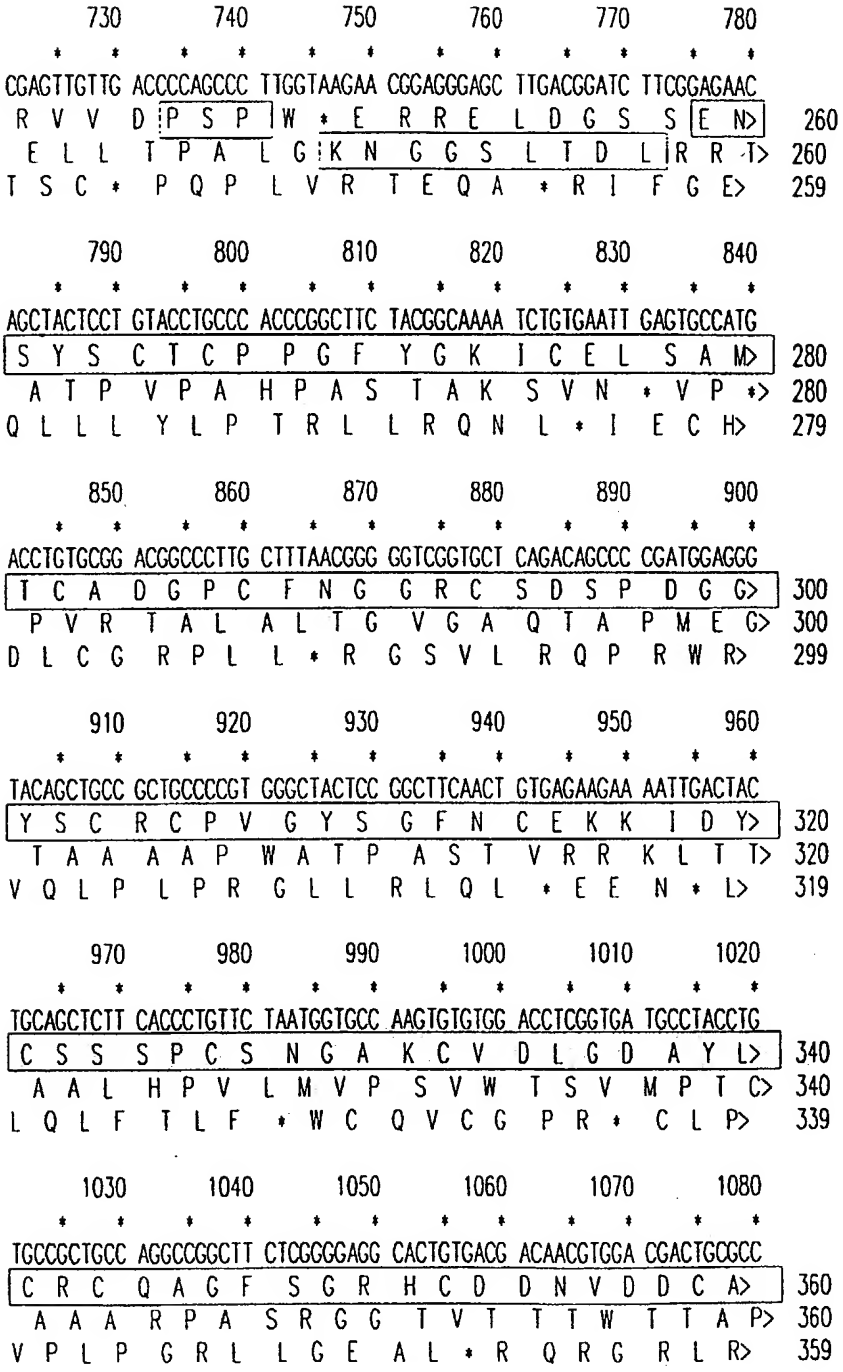
P W L E R A L L H R A D L P A W M * * A> 140
P G W K G P Y C T E P I C L P G C D E Q> 140
T L A G K G P T A Q S R S A C L D V M S> 139
430 440 450 460 470 480
GCATGGATTT TGTGACAAAC CAGCCCAATG CAAGTGCAGA GTGGGCTGGC AGGGCCGGTA
A W I L * Q T R G M Q V Q S G L A G P V> 160
H G F C D K P G E C K C R V G W Q G R Y> 160
S M D F V T N Q G N A S A E W A G R A G> 159
490 500 510 520 530 540
CTGTGACGAG TGTATCCGCT ATCCAGGCTG TCTCCATGGC ACCTGCCAGC AGCCCTGGCA
L * R V Y P L S R L S P W H L P A A L A> 180
C D E C I R Y P G C L H G T C Q Q P W Q> 180
T V T S V S A I Q A V S M A P A S S P G> 179
550 560 570 580 590 600
GTGCAACTGC CAGGAAGGNT GGGGGGGCCT TTTCTGCAAC CAGGACCTGA ACTACTGCAC
V Q L P G R X G G P F L Q P G P E L L H> 200
C N C Q E G W G G L F C N Q D L N Y C T> 200
S A T A R K X G G A F S A T R T * T T A> 199
610 620 630 640 650 660
ACACCATAAG CCCTGCAAGA ATCGAGCCAC CTGCAACAAA CACGGGCCAG GGGGAGCTAC
T P * A L Q E W S H L Q Q T R A R G S Y> 220
H H K P C K N G A T C N K H G P G G A T> 220
H T I S P A R M E P P A T N T G Q G E L> 219
670 680 690 700 710 720
ACTTGGTCTT TGGCCGGNCT GGGGTACANA GGGTGCCACC TGCGAAGCTT GGGGATTGGA
T W S L A G L G Y X G C H L R S L G I G> 240
L G L W P X W G T X G A T C E A W G L D> 240
H L V F G R X C V X R V P P A K L G D W> 239

FIG.12B2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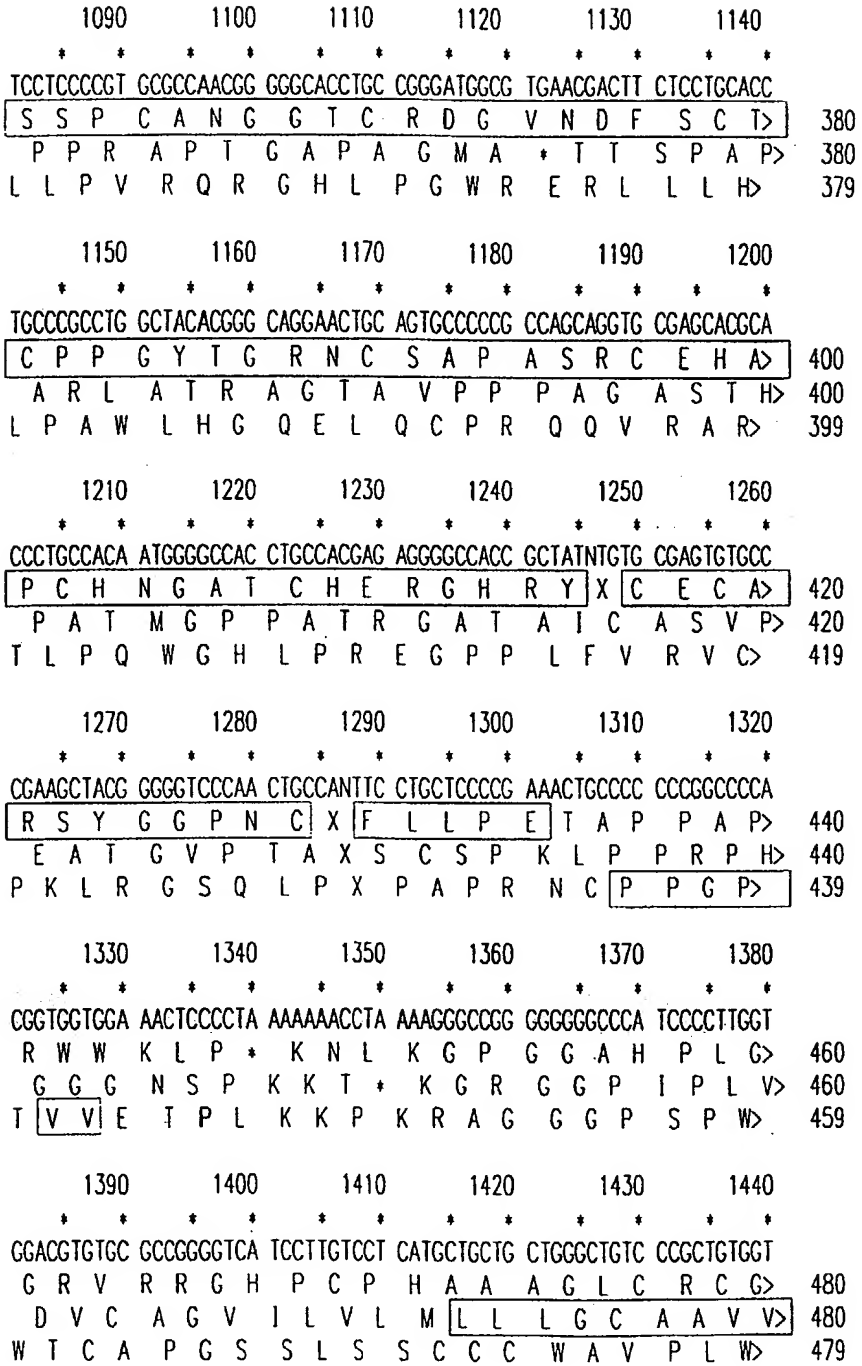
E L L T P A L G K N G G S L T D L R R T> 260
T S C * P Q P L V R T E Q A * R I F G E> 259
790 800 810 820 830 840
AGCTACTCCT GTACCTGCCC ACCCGGCTTC TACGGCAAAA TCTGTGAATT GAGTGCCATG
S Y S C T C P P G F Y G K I C E L S A M> 280
A T P V P A H P A S T A K S V N * V P *> 280
Q L L L Y L P T R L L R Q N L * I E C H> 279
850 860 870 880 890 900
ACCTGTGCGG ACGGCCCTTG CTTTAACGGG GGTCGGTGCT CAGACAGCCC CGATGGAGGG
T C A D G P C F N G G R C S D S P D G G> 300
P V R T A L A L T G V G A Q T A P M E G> 300
D L C G R P L L * R G S V L R Q P R W R> 299
910 920 930 940 950 960
TACAGCTGCC GCTGCCCCGT GGGCTACTCC GGCTTCAACT GTGAGAAGAA AATTGACTAC
Y S C R C P V G Y S G F N C E K K I D Y> 320
T A A A A P W A T P A S T V R R K L T T> 320
V Q L P L P R G L L R L Q L * E E N * L> 319
970 980 990 1000 1010 1020
TGCAGCTCTT CACCCTGTTC TAATGGTGCC AAGTGTGTGG ACCTCGGTGA TGCCTACCTG
C S S S P C S N G A K C V D L G D A Y L> 340
A A L H P V L M V P S V W T S V M P T C> 340
L Q L F T L F * W C Q V C G P R * C L P> 339
1030 1040 1050 1060 1070 1080
TGCCGCTGCC AGGCCGGCTT CTCGGGGAGG CACTGTGACG ACAACGTGGA CGACTGCGCC
C R C Q A G F S G R H C D D N V D D C A> 360
A A A R P A S R G G T V T T T W T T A P> 360
V P L P G R L L G E A L * R Q R G R L R> 359

FIG.12B3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S S P C A N G G T C R D G V N D F S C T> 380
P P R A P T G A P A G M A * T T S P A P> 380
L L P V R Q R G H L P G W R E R L L L H> 379
1150 1160 1170 1180 1190 1200
TGCCCGCCTG GCTACACGGG CAGGAACTGC AGTGCCCCCG CCAGCAGGTG CGAGCACGCA
C P P G Y T G R N C S A P A S R C E H A> 400
A R L A T R A G T A V P P P A G A S T H> 400
L P A W L H G Q E L Q C P R Q Q V R A R> 399
1210 1220 1230 1240 1250 1260
CCCTGCCACA ATGGGGCCAC CTGCCACGAG AGGGGCCACC GCTATNTGTG CGAGTGTGCC
P C H N G A T C H E R G H R Y X C E C A> 420
P A T M G P P A T R G A T A I C A S V P> 420
T L P Q W G H L P R E G P P L F V R V C> 419
1270 1280 1290 1300 1310 1320
CGAAGCTACG GGGGTCCCAA CTGCCANTTC CTGCTCCCCG AAACTGCCCC CCCGGCCCCA
R S Y G G P N C X F L L P E T A P P A P> 440
E A T G V P T A X S C S P K L P P R P H> 440
P K L R G S Q L P X P A P R N C P P G P> 439
1330 1340 1350 1360 1370 1380
CGGTGGTGGA AACTCCCCTA AAAAAACCTA AAAGGGCCCG GGGGGGCCCA TCCCCTTGGT
R W W K L P * K N L K G P G G A H P L G> 460
G G G N S P K K T * K G R G G P I P L V> 460
T V V E T P L K K P K R A G G G P S P W> 459
1390 1400 1410 1420 1430 1440
GGACGTGTGC GCCGGGGTCA TCCTTGTCCT CATGCTGCTG CTGGGCTGTC CCGCTGTGGT
G R V R R G H P C P H A A A G L C R C G> 480
D V C A G V I L V L M L L L G C A A V V> 480
W T C A P G S S L S S C C C W A V P L W> 479

FIG.12B4

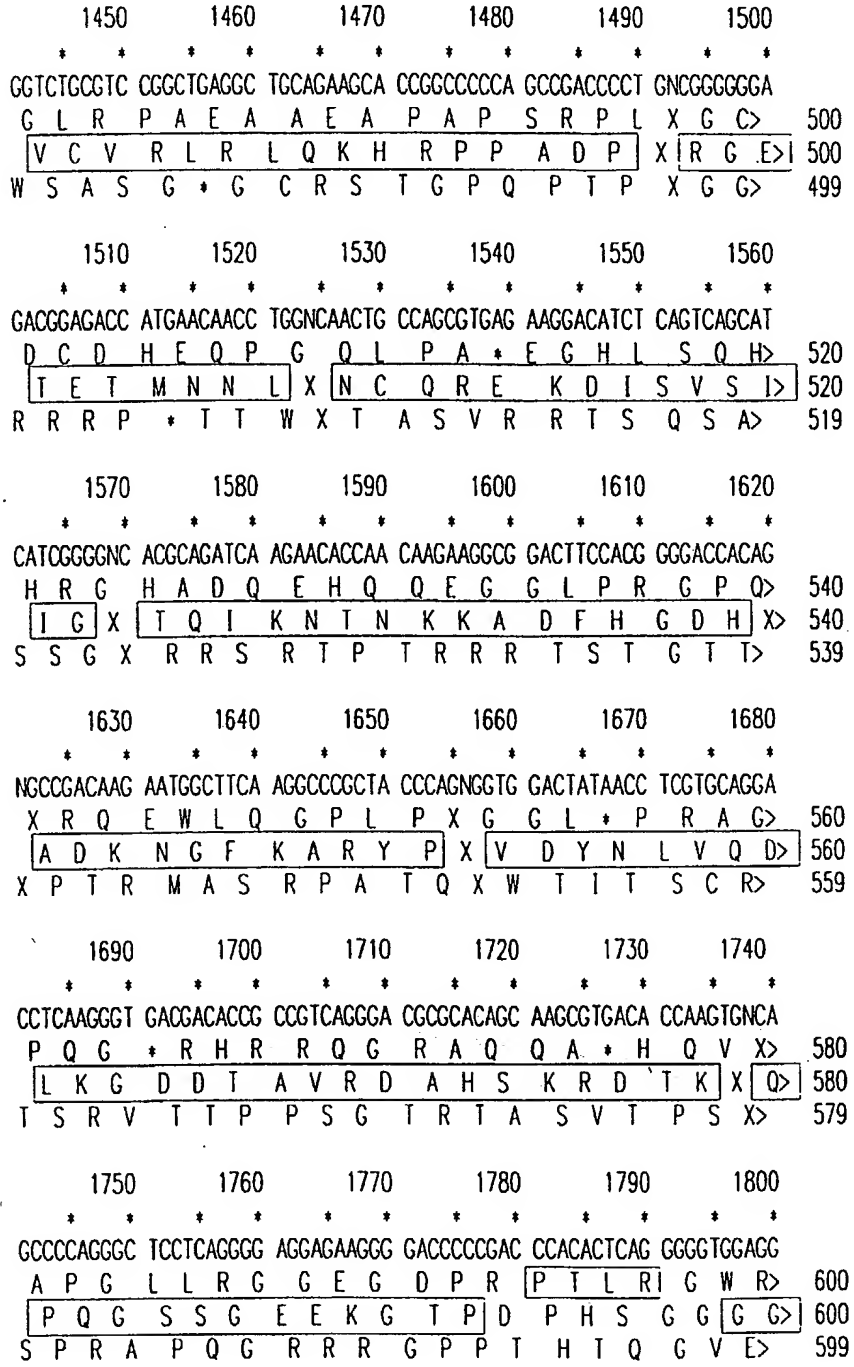
1450 1460 1470 1480 1490 1500
GGTCTGCGTC CGGCTGAGGC TGCAGAAGCA CCGGCCCCCA GCCGACCCCT GNCGGGGGGA
G L R P A E A A E A P A P S R P L X G C> 500
V C V R L R L Q K H R P P A D P X R G E> 500
W S A S G * G C R S T G P Q P T P X G G> 499
1510 1520 1530 1540 1550 1560
GACGGAGACC ATGAACAACC TGGNCAACTG CCAGCGTGAG AAGGACATCT CAGTCAGCAT
D C D H E Q P G Q L P A * E G H L S Q H> 520
T E T M N N L X N C Q R E K D I S V S I> 520
R R R P * T T W X T A S V R R T S Q S A> 519
1570 1580 1590 1600 1610 1620
CATCGGGGNC ACGCAGATCA AGAACACCAA CAAGAAGGCG GACTTCCACG GGGACCACAG
H R G H A D Q E H Q Q E G G L P R G P Q> 540
I G X T Q I K N T N K K A D F H G D H X> 540
S S G X R R S R T P T R R R T S T G T T> 539
1630 1640 1650 1660 1670 1680
NGCCGACAAG AATGGCTTCA AGGCCCGCTA CCCAGNGGTG GACTATAACC TCGTGCAGGA
X R Q E W L Q G P L P X G G L * P R A G> 560
A D K N G F K A R Y P X V D Y N L V Q D> 560
X P T R M A S R P A T Q X W T I T S C R> 559
1690 1700 1710 1720 1730 1740
CCTCAAGGGT GACGACACCG CCGTCAGGGA CGCGCACAGC AAGCGTGACA CCAAGTGNCA
P Q G * R H R R Q G R A Q Q A * H Q V X> 580
L K G D D T A V R D A H S K R D T K X Q> 580
T S R V T T P P S G T R T A S V T P S X> 579
1750 1760 1770 1780 1790 1800
GCCCCAGGGC TCCTCAGGGG AGGAGAAGGG GACCCCCGAC CCACACTCAG GGGGTGGAGG
A P G L L R G G E G D P R P T L R I G W R> 600
P Q G S S G E E K G T P D P H S G G G G> 600
S P R A P Q G R R R G P P T H T Q G V E> 599

FIG.12B5

```
     1810       1820       1830       1840       1850       1860
      *    *     *    *     *    *     *    *     *    *     *    *
AAGCATCTTG AAAGAAAAAG GCCGGACTTC GGGCTTGTTC AACTTTCAAA AGACAANCAA
 K  H  L   E [R  K  R  P] D  F   G  L  V   Q  L [S  K  D] X  Q>   620
  S [I] L   K  E  K   G  R  T [S] G  L  F   N  F  Q   K [T] X  X>  620
E  A  S  *   K  K  K   A  G  L   R  A  C [S  T] F   K  R  Q  X>   619

     1870       1880       1890       1900       1910       1920
      *    *     *    *     *    *     *    *     *    *     *    *
NGTACAAGTC GGTGTNCGTC ATTTCCGNAG GAGGAAGGNT GACTGCGTCA TAGGAANTTG
 X  T  S   R  C  X   S  F  P   X  E  E   G  *  L   R  H  R   X  L>   640
  V  Q  V   G  V  R   H  F  R   R  R  K   X  D [C  V  I] G   X  *>  640
X  Y  K   S  V  X   V  I  S   X  G  G   R  X  T   A  S  *   E  X>   639

     1930       1940       1950       1960       1970       1980
      *    *     *    *     *    *     *    *     *    *     *    *
AGGTNGTAAA NTGGNAGTTG ANNTTGGAAA GNNNTCCCCC GATTCCCNTT TCAAAGTTTT
 R  X  *   X  G  S   *  X  W  K   X  X  P   G  F  R   F  Q  S  F>   660
  G  X  K   X  X  V   X  X  G  K   X  S  P   D  S  X   F  K  V  F>  660
[E  V] V   X  W  X   L  X  L  E   X  X  P   R  I  P   X  S  K  F>   659
```

FIG.12B6

```
MOUSE DELTA DNA  GTCCAGCGGT ACCATGGGCC GTCGGAGCGC GCTAGCCCTT GCCGTGGTCT   50
HUMAN DELTA      ---------- ---------- ---------- ---------- ----------

CONSENSUS        GTCCAGCGGT ACCATGGGCC GTCGGAGCGC GCTAGCCCTT GCCGTGGTCT   50

MOUSE DELTA DNA  CTGCCCTGCT GTGCCAGGTC TGGAGCTCCG GCGTATTTGA GCTGAAGCTG  100
HUMAN DELTA      ---------- ---------- ---------- ---------- ----------

CONSENSUS        CTGCCCTGCT GTGCCAGGTC TGGAGCTCCG GCGTATTTGA GCTGAAGCTG  100

MOUSE DELTA DNA  CAGGAGTTCG TCAACAAGAA GGGGCTGCTG GGGAACCGCA ACTGCTGCCG  150
HUMAN DELTA      ---------- ---------- ---------- ---------- ----------

CONSENSUS        CAGGAGTTCG TCAACAAGAA GGGGCTGCTG GGGAACCGCA ACTGCTGCCG  150

MOUSE DELTA DNA  CGGGGGCTCT GGCCCGCCTT GCGCCTGCAG GACCTTCTTT CGCGTATGCC  200
HUMAN DELTA      ---------- ---------- ---------- ---------- ----------

CONSENSUS        CGGGGGCTCT GGCCCGCCTT GCGCCTGCAG GACCTTCTTT CGCGTATGCC  200

MOUSE DELTA DNA  TCAAGCACTA CCAGGCCAGC GTGTCACCGG AGCCACCCTG CACCTACGGC  250
HUMAN DELTA      ---------- ---------- ---------- ---------- ----------

CONSENSUS        TCAAGCACTA CCAGGCCAGC GTGTCACCGG AGCCACCCTG CACCTACGGC  250

MOUSE DELTA DNA  AGTGCTGTCA CGCCAGTGCT GGGTGTCGAC TCCTTCAGCC TGCCTGATGG  300
HUMAN DELTA      ---------- ---------- ---------- ---------- -----CATTG    5

CONSENSUS        AGTGCTGTCA CGCCAGTGCT GGGTGTCGAC TCCTTCAGCC TGCCTSATKG  300

MOUSE DELTA DNA  GGCAGGCATC GACCCC---G CCTTCAGCAA CCCCA--TCC GATTTC-CCC  343
HUMAN DELTA      GGTACGGGCC CCCCTCGAGG TCGACGGTAT CGATAAGCTT GATATCGAAT   55

CONSENSUS        SGYASGSRYC SMCCYCGAGG YCKWCRGYAW CSMYAAGYYY GATATCGMMY  350

MOUSE DELTA DNA  TTCGGCTTCA CCTGGCCAGG TACCTTCTCT CTGATCATTG AAGCCCTCCA  393
HUMAN DELTA      TCCGGCTTCA CCTGGCCGGG CACCTTCTCT CTGATTATTG AAGCTCTCCA  105

CONSENSUS        TYCGGCTTCA CCTGGCCRGG YACCTTCTCT CTGATYATTG AAGCYCTCCA  400

MOUSE DELTA DNA  TACAGACTCT CCCGATGACC TCGCAACAGA AAACCCAGAA AGACTCATCA  443
HUMAN DELTA      CACAGATTCT CCTGATGACC TCGCAACAGA AAACCCAGAA AGACTCATCA  155

CONSENSUS        YACAGAYTCT CCYGATGACC TCGCAACAGA AAACCCAGAA AGACTCATCA  450
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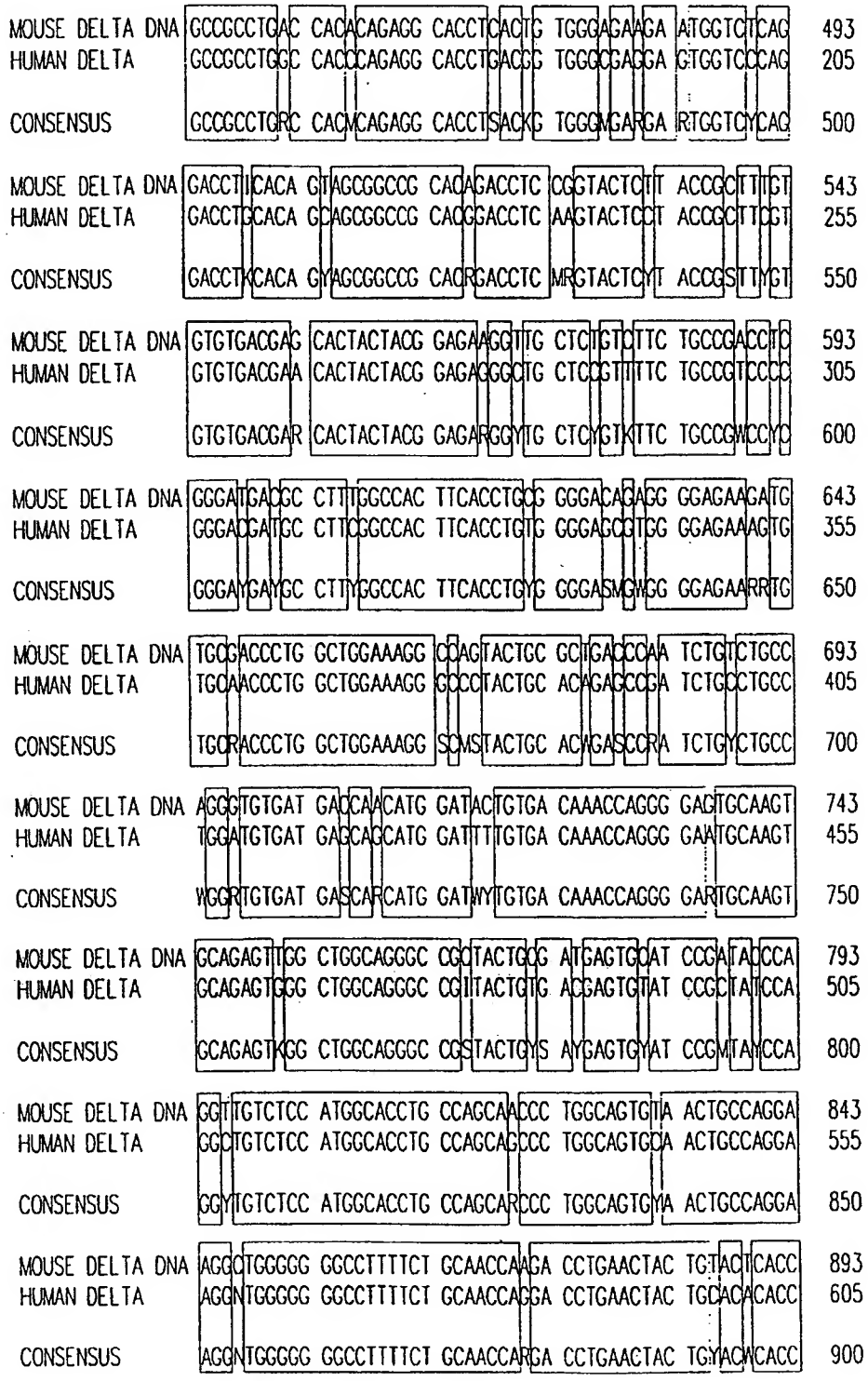
FIG.13A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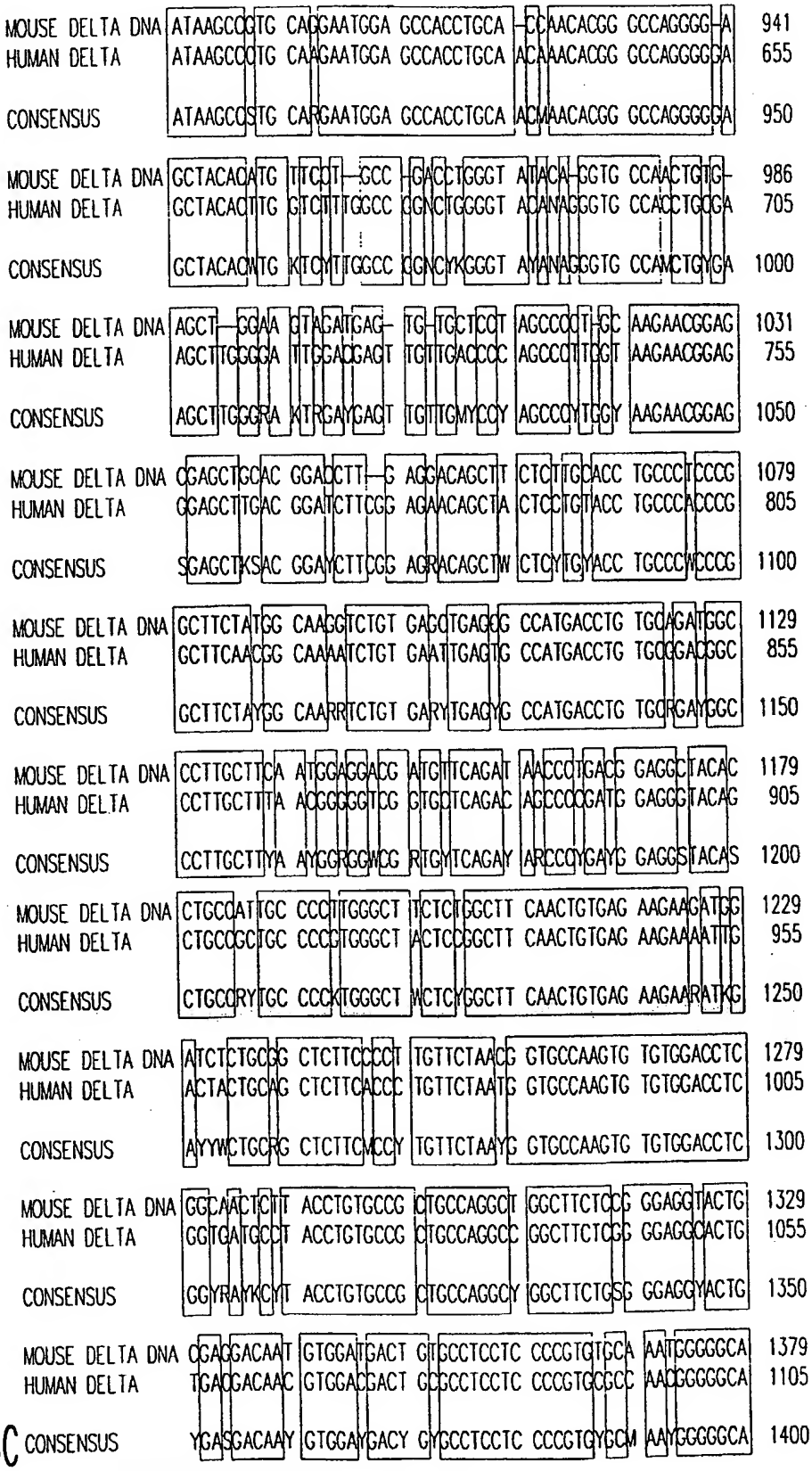
HUMAN DELTA GCCGCCTGGC CACCCAGAGG CACCTGACGG TGGGCGAGGA GTGGTCCCAG 205
CONSENSUS GCCGCCTGRC CACMCAGAGG CACCTSACKG TGGGMGARGA RTGGTCYCAG 500
MOUSE DELTA DNA GACCTTCACA GTAGCGGCCG CACAGACCTC CGGTACTCTT ACCGCTTTGT 543
HUMAN DELTA GACCTGCACA GCAGCGGCCG CACGGACCTC AAGTACTCCT ACCGCTTCGT 255
CONSENSUS GACCTKCACA GYAGCGGCCG CACRGACCTC MRGTACTCYT ACCGSTTYGT 550
MOUSE DELTA DNA GTGTGACGAG CACTACTACG GAGAAGGTTG CTCTGTCTTC TGCCGACCTC 593
HUMAN DELTA GTGTGACGAA CACTACTACG GAGAGGGCTG CTCCGTTTTC TGCCGTCCCC 305
CONSENSUS GTGTGACGAR CACTACTACG GAGARGGYTG CTCYGTKTTC TGCCGWCCYC 600
MOUSE DELTA DNA GGGATGACGC CTTTGGCCAC TTCACCTGCG GGGACAGAGG GGAGAAGATG 643
HUMAN DELTA GGGACGATGC CTTCGGCCAC TTCACCTGTG GGGAGCGTGG GGAGAAAGTG 355
CONSENSUS GGGAYGAYGC CTTYGGCCAC TTCACCTGYG GGGASMGWGG GGAGAARRTG 650
MOUSE DELTA DNA TGCGACCCTG GCTGGAAAGG CCAGTACTGC GCTGACCCAA TCTGTCTGCC 693
HUMAN DELTA TGCAACCCTG GCTGGAAAGG GCCCTACTGC ACAGAGCCGA TCTGCCTGCC 405
CONSENSUS TGCRACCCTG GCTGGAAAGG SCMSTACTGC ACWGASCCRA TCTGYCTGCC 700
MOUSE DELTA DNA AGGGTGTGAT GACCAACATG GATACTGTGA CAAACCAGGG GAGTGCAAGT 743
HUMAN DELTA TGGATGTGAT GAGCAGCATG GATTTTGTGA CAAACCAGGG GAATGCAAGT 455
CONSENSUS WGGRTGTGAT GASCARCATG GATWYTGTGA CAAACCAGGG GARTGCAAGT 750
MOUSE DELTA DNA GCAGAGTTGG CTGGCAGGGC CGCTACTGCG ATGAGTGCAT CCGATACCCA 793
HUMAN DELTA GCAGAGTGGG CTGGCAGGGC CGTTACTGTG ACGAGTGTAT CCGCTATCCA 505
CONSENSUS GCAGAGTKGG CTGGCAGGGC CGSTACTGYS AYGAGTGYAT CCGMTAYCCA 800
MOUSE DELTA DNA GGTTTGTCTCC ATGGCACCTG CCAGCAACCC TGGCAGTGTA ACTGCCAGGA 843
HUMAN DELTA GGCTGTCTCC ATGGCACCTG CCAGCAGCCC TGGCAGTGCA ACTGCCAGGA 555
CONSENSUS GGYTGTCTCC ATGGCACCTG CCAGCARCCC TGGCAGTGYA ACTGCCAGGA 850
MOUSE DELTA DNA AGGCTGGGGG GGCCTTTTCT GCAACCAAGA CCTGAACTAC TGTACTCACC 893
HUMAN DELTA AGGNTGGGGG GGCCTTTTCT GCAACCAGGA CCTGAACTAC TGCACACACC 605
CONSENSUS AGGNTGGGGG GGCCTTTTCT GCAACCARGA CCTGAACTAC TGYACWCACC 900

FIG.13B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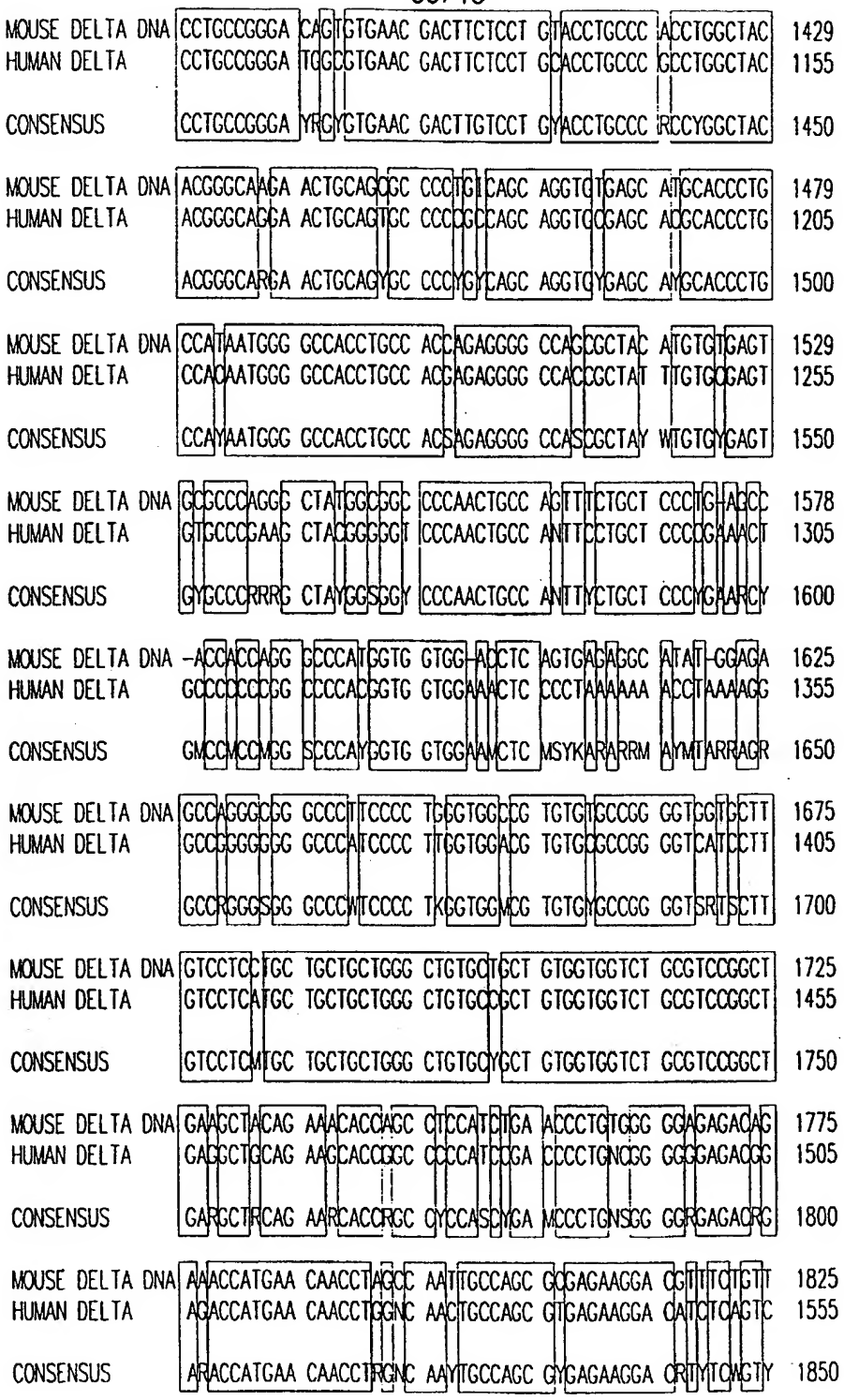
HUMAN DELTA ATAAGCCCTG CAAGAATGGA GCCACCTGCA ACAAACACGG GCCAGGGGGA 655
CONSENSUS ATAAGCCSTG CARGAATGGA GCCACCTGCA ACMAACACGG GCCAGGGGGA 950
MOUSE DELTA DNA GCTACACATG TTCCCT-GCC -GACCTGGGT ATTACA-GGTG CCAACTGTGG- 986
HUMAN DELTA GCTACACTTG GTCTTTTGCCC CGNCTGGGGT ACANAGGGTG CCACCTGCGA 705
CONSENSUS GCTACACWTG KTCYTTGGCC CGNCYKGGGT AMANAGGGTG CCAMCTGYGA 1000
MOUSE DELTA DNA AGCT-GGAA GTAGATGAG- TG-TGCTCCT AGCCCCT-GC AAGAACGGAG 1031
HUMAN DELTA AGCTTGGGGA TTGGACGAGT TGTTGACCCC AGCCCTTGGT AAGAACGGAG 755
CONSENSUS AGCTTGGGRA KTRGAYGAGT TGTTGMYCCY AGCCCYTGGY AAGAACGGAG 1050
MOUSE DELTA DNA CGAGCTGCAC GGACCTT-G AGGACAGCTT CTCTTGCACC TGCCCTCCCG 1079
HUMAN DELTA GGAGCTTGAC GGATCTTCGG AGAACAGCTA CTCCTGTACC TGCCCACCCG 805
CONSENSUS SGAGCTKSAC GGAYCTTCGG AGRACAGCTW CTCYTGYACC TGCCCWCCCG 1100
MOUSE DELTA DNA GCTTCTATGG CAAGGTCTGT GAGCTGAGGG CCATGACCTG TGCAGATGGC 1129
HUMAN DELTA GCTTCAACGG CAAAATCTGT GAATTGAGTG CCATGACCTG TGCGGACGGC 855
CONSENSUS GCTTCTAYGG CAARRTCTGT GARYTGAGYG CCATGACCTG TGCRGAYGGC 1150
MOUSE DELTA DNA CCTTGCTTCA ATGGAGGACG ATGTTCAGAT AACCCTGACG GAGGCTACAC 1179
HUMAN DELTA CCTTGCTTTA ACGGGGGTCG GTGCTCAGAC AGCCCCGATG GAGGGTACAG 905
CONSENSUS CCTTGCTTYA AYGGRGGWCG RTGYTCAGAY ARCCCYGAYG GAGGSTACAS 1200
MOUSE DELTA DNA CTGCCATTGC CCCTTGGGCT TCTCTGGCTT CAACTGTGAG AAGAAGATGG 1229
HUMAN DELTA CTGCCGCTGC CCCGTGGGCT ACTCCGGCTT CAACTGTGAG AAGAAAATTG 955
CONSENSUS CTGCCRYTGC CCCKTGGGCT WCTCYGGCTT CAACTGTGAG AAGAARATKG 1250
MOUSE DELTA DNA ATCTCTGCGG CTCTTCCCCT TGTTCTAACG GTGCCAAGTG TGTGGACCTC 1279
HUMAN DELTA ACTACTGCAG CTCTTCACCC TGTTCTAATG GTGCCAAGTG TGTGGACCTC 1005
CONSENSUS AYYWCTGCRG CTCTTCMCCY TGTTCTAAYG GTGCCAAGTG TGTGGACCTC 1300
MOUSE DELTA DNA GGCAACTCTT ACCTGTGCCG CTGCCAGGCT GGCTTCTCCG GGAGGTACTG 1329
HUMAN DELTA GGTGATGCCT ACCTGTGCCG CTGCCAGGCC GGCTTCTCGG GGAGGCACTG 1055
CONSENSUS GGYRAMKCYT ACCTGTGCCG CTGCCAGGCY GGCTTCTCSG GGAGGYACTG 1350
MOUSE DELTA DNA CGACGACAAT GTGGATGACT GTGCCTCCTC CCCGTGTGCA AATGGGGGCA 1379
HUMAN DELTA TGACGACAAC GTGGACGACT GCGCCTCCTC CCCGTGCGCC AACGGGGGCA 1105
CONSENSUS YGASGACAAY GTGGAYGACY GYGCCTCCTC CCCGTGYGCM AAYGGGGGCA 1400

FIG.13C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HUMAN DELTA CCTGCCGGGA TGGCGTGAAC GACTTCTCCT GCACCTGCCC GCCTGGCTAC 1155
CONSENSUS CCTGCCGGGA YRGYGTGAAC GACTTGTCCT GYACCTGCCC RCCYGGCTAC 1450
MOUSE DELTA DNA ACGGGCAAGA ACTGCAGCGC CCCTGTCAGC AGGTGTGAGC ATGCACCCTG 1479
HUMAN DELTA ACGGGCAGGA ACTGCAGTGC CCCCGCCAGC AGGTGCGAGC ACGCACCCTG 1205
CONSENSUS ACGGGCARGA ACTGCAGYGC CCCYGYCAGC AGGTGYGAGC AYGCACCCTG 1500
MOUSE DELTA DNA CCATAATGGG GCCACCTGCC ACCAGAGGGG CCAGCGCTAC ATGTGTGAGT 1529
HUMAN DELTA CCACAATGGG GCCACCTGCC ACGAGAGGGG CCACCGCTAT TTGTGCGAGT 1255
CONSENSUS CCAYAATGGG GCCACCTGCC ACSAGAGGGG CCASCGCTAY WTGTGYGAGT 1550
MOUSE DELTA DNA GCGCCCAGGG CTATGGCGGC CCCAACTGCC AGTTTCTGCT CCCTG-AGCC 1578
HUMAN DELTA GTGCCCGAAG CTACGGGGGT CCCAACTGCC ANTTCCTGCT CCCCGAAACT 1305
CONSENSUS GYGCCCRRRG CTAYGGSGGY CCCAACTGCC ANTTYCTGCT CCCYGAARCY 1600
MOUSE DELTA DNA -ACCACCAGG GCCCATGGTG GTGG-ACCTC AGTGAGAGGC ATAT-GGAGA 1625
HUMAN DELTA GCCCCCCCGG CCCCACGGTG GTGGAAACTC CCCTAAAAAAA ACCTAAAAGG 1355
CONSENSUS GMCCMCCMGG SCCCAYGGTG GTGGAAMCTC MSYKARARRM AYMTARRAGR 1650
MOUSE DELTA DNA GCCAGGGCGG GCCCTTCCCC TGGGTGGCCG TGTGTGCCGG GGTGGTGCTT 1675
HUMAN DELTA GCCGGGGGGG GCCCATCCCC TTGGTGGACG TGTGCGCCGG GGTCATCCTT 1405
CONSENSUS GCCRGGGSGG GCCCWTCCCC TKGGTGGMCG TGTGYGCCGG GGTSRTSCTT 1700
MOUSE DELTA DNA GTCCTCCTGC TGCTGCTGGG CTGTGCTGCT GTGGTGGTCT GCGTCCGGCT 1725
HUMAN DELTA GTCCTCATGC TGCTGCTGGG CTGTGCCGCT GTGGTGGTCT GCGTCCGGCT 1455
CONSENSUS GTCCTCMTGC TGCTGCTGGG CTGTGCYGCT GTGGTGGTCT GCGTCCGGCT 1750
MOUSE DELTA DNA GAAGCTACAG AAACACCAGC CTCCATCTGA ACCCTGTGGG GGAGAGACAG 1775
HUMAN DELTA GAGGCTGCAG AAGCACCGGC CCCCATCCGA CCCCTGNCGG GGGGAGACGG 1505
CONSENSUS GARGCTRCAG AARCACCRGC CYCCASCYGA MCCCTGNSGG GGRGAGACRG 1800
MOUSE DELTA DNA AAACCATGAA CAACCTAGCC AATTGCCAGC GCGAGAAGGA CGTTTTCTGTT 1825
HUMAN DELTA AGACCATGAA CAACCTGGNC AACTGCCAGC GTGAGAAGGA CATCTCAGTC 1555
CONSENSUS ARACCATGAA CAACCTRGNC AAYTGCCAGC GYGAGAAGGA CRTYTCWGTY 1850

FIG.13D

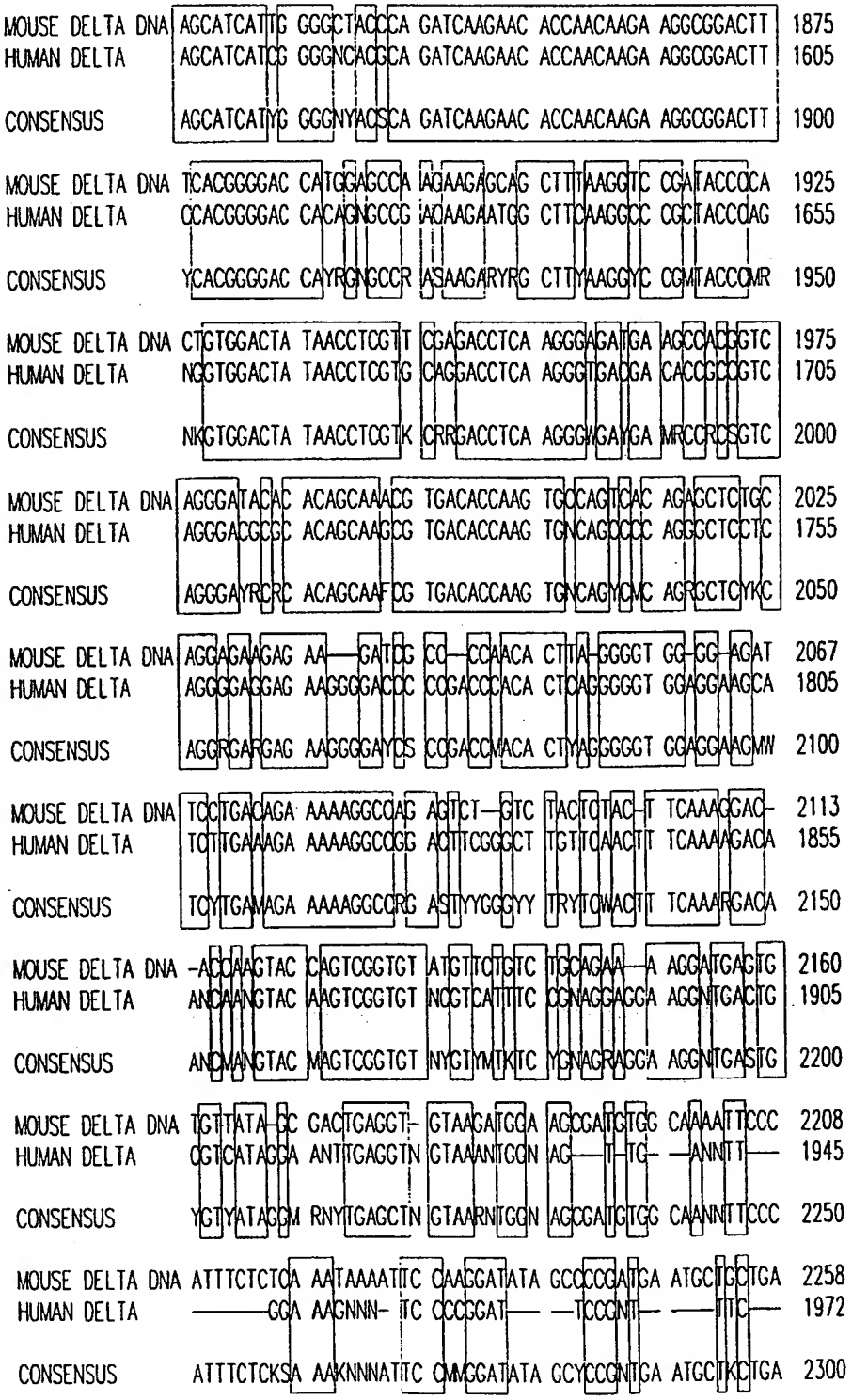

FIG. 13E

SUBSTITUTE SHEET (RULE 26)

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MOUSE DELTA DNA GAGAGGAAGG GAGAGGAAAC CCAGGGACTG CTGCTGAGAA CCAGGTTCAG  2308
HUMAN DELTA     ---------- ------AAA- ---------G TTTTTT----- ----------  1981

CONSENSUS       GAGAGGAAGG GAGAGGAAAC CCAGGGACTG YTKYTCAGAA CCAGGTTCAG  2350

MOUSE DELTA DNA GCGAAGCTGG TTCTCTCAGA GTTAGCAGAG GCGCCCGACA CTGCCAGCCT  2358
HUMAN DELTA     ---------- ---------- ---------- ---------- ----------  1981

CONSENSUS       GCGAAGCTGG TTCTCTCAGA GTTAGCAGAG GCGCCCGACA CTGCCAGCCT  2400

MOUSE DELTA DNA AGGCTTTGGC TGCCGCTGGA CTGCCTGCTG GTTGTTCCCA TTGCACTATG  2408
HUMAN DELTA     ---------- ---------- ---------- ---------- ----------  1981

CONSENSUS       AGGCTTTGGC TGCCGCTGGA CTGCCTGCTG GTTGTTCCCA TTGCACTATG  2450

MOUSE DELTA DNA GACAGTTGCT TTGAAGAGTA TATATTTAAA TGGACGAGTG ACTTGATTCA  2458
HUMAN DELTA     ---------- ---------- ---------- ---------- ----------  1981

CONSENSUS       GACAGTTGCT TTGAAGAGTA TATATTTAAA TGGACGAGTG ACTTGATTCA  2500

MOUSE DELTA DNA TATAGGAAGC ACGCACTGCC CACACGTCTA TCTTGGATTA CTATGAGCCA  2508
HUMAN DELTA     ---------- ---------- ---------- ---------- ----------  1981

CONSENSUS       TATAGGAAGC ACGCACTGCC CACACGTCTA TCTTGGATTA CTATGAGCCA  2550

MOUSE DELTA DNA GTCTTTCCTT GAACTAGAAA CACAACTGCC TTTATTGTCC TTTTTGATAC  2558
HUMAN DELTA     ---------- ---------- ---------- ---------- ----------  1981

CONSENSUS       GTCTTTCCTT GAACTAGAAA CACAACTGCC TTTATTGTCC TTTTTGATAC  2600

MOUSE DELTA DNA TGAGATGTGT TTTTTTTTTT CCTAGACGGG AAAAAGAAAA CGTGTGTTAT  2608
HUMAN DELTA     ---------- ---------- ---------- ---------- ----------  1981

CONSENSUS       TGAGATGTGT TTTTTTTTTT CCTAGACGGG AAAAAGAAAA CGTGTGTTAT  2650

MOUSE DELTA DNA TTTTTTGGGA TTTGTAAAAA TATTTTTCAT GATATCTGTA AAGCTTGAGT  2658
HUMAN DELTA     ---------- ---------- ---------- ---------- ----------  1981

CONSENSUS       TTTTTTGGGA TTTGTAAAAA TATTTTTCAT GATATCTGTA AAGCTTGAGT  2700

MOUSE DELTA DNA ATTTTGTGAC GTTCATTTTT TTATAATTTA AATTTTGGTA AATATGTACA  2708
HUMAN DELTA     ---------- ---------- ---------- ---------- ----------  1981

CONSENSUS       ATTTTGTGAC GTTCATTTTT TTATAATTTA AATTTTGGTA AATATGTACA  2750
```

FIG.13F

```
MOUSE DELTA DNA AAGGCACTTC GGGTCTATGT GACTATATTT TTTTGTATAT AAATGTATTT  2758
HUMAN DELTA     ---------- ---------- ---------- ---------- ----------  1981

CONSENSUS       AAGGCACTTC GGGTCTATGT GACTATATTT TTTTGTATAT AAATGTATTT  2800


MOUSE DELTA DNA ATGGAATATT GTGCAAATGT TATTTGAGTT TTTTACTGTT TTGTTAATGA  2808
HUMAN DELTA     ---------- ---------- ---------- ---------- ----------  1981

CONSENSUS       ATGGAATATT GTGCAAATGT TATTTGAGTT TTTTACTGTT TTGTTAATGA  2850

MOUSE DELTA DNA AGAAATTCAT TTTAAAAATA TTTTTCCAAA ATAAATATAA TGAACTACA  2857
HUMAN DELTA     ---------- ---------- ---------- ---------- ----------  1981

CONSENSUS       AGAAATTCAT TTTAAAAATA TTTTTCCAAA ATAAATATAA TGAACTACA  2899
```

FIG.13G

```
GFTWPGTFSLIIEALHTDSPD>      21
DLATENPERLISRLATQRHL>       41
TVGEEWSQDLHSSGRIDLKY>       61
SYRFVCDEHYYGEGCSVFCR>       81
PRDDAFGHFTCGERGEKVCN>      101
PGWKGPYCTEPICLPGCDEQ>      121
HGFCDKPGECKCRVGWQGRY>      141
CDECIRYPGCLHGTCQQPWQ>      161
CNCQEGWGGLFCNQDLNYCT>      181
HHKPCKNGAIC*TNTGQG*        198
SYT*PSP*KNGGSLTDL*         213
ENSYSCTCPPGFYGKICELSAM>    235
TCADGPCFNGGRCSDSPDGG>      255
YSCRCPVGYSGFNCEKKIDY>      275
CSSSPCSNGAKCVDLGDAYL>      295
CRCQAGFSGRHCDDNVDDCA>      315
SSPCANGGTCRDGVNDFSCT>      335
CPPGYTGRNCSAPASRCEHA>      355
PCHNGATCHERGHRY*CECA>      374
RSYGGPNC*FLLPE*PPGP*>      391
VV*LLLGCAAVVVCVRLRLQKH>    412
RPPADP*RGETETMNNL*>        428
```

FIG. 14A

NCQREKDISVSIIG*TQIKNTN> 449

KKADFHGDH*ADKNGFKARYP* 469

VDYNLVQDLKGDDTAVRDAHSKRDTK* 494

QPQGSSGEEKGTP*PTLR*GG* 514

I*RKRP*S*ST*SKD*T* 526

CVI*EV* 531

FIG. 14B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/11178

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 17/00; C07K 14/00; C12P 21/06; C12N 5/00, 15/00
US CL :536/23.1; 530/350; 435/69.1, 320.1, 240.1; 514/12, 44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1; 530/350; 435/69.1, 320.1, 240.1; 514/12, 44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

None

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KOPCZYNSKI et al. Delta, a Drosophila neurogenic gene, is transcriptionally complex and encodes a protein related to blood coagulation factors and epidermal growth factor of vertebrates. Genes & Development. December 1988, Vol. 2, pages 1723-1735, see entire document.	12, 13, 39, 56
Y	VASSIN et al. The neurogenic gene Delta of Drosophila melangaster is expressed in neurogenic territories and encodes a putative transmembrane protein with EGF-like repeats. EMBO Journal, 1987, Vol. 6, No. 11, pages 3431-3440, see entire document.	12, 13, 39, 56

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 SEPTEMBER 1996	Date of mailing of the international search report 31 OCT 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Karen Cochrane Carlson, Ph.D. Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/11178

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/11178

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

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Group I, claim(s)s 1-28, 49-56, 75-81, 84-89, 93, 94, drawn to vertebrate Delta protein.

Group II, claim(s) 29-32, 60, and 61, drawn to antibodies against vertebrate Delta.

Group III, claim(s) 33-48, 57-59, 70, 71, 82, 83, 90-92, 95, 96, 98, drawn to DNA encoding vertebrate Delta.

Group IV, claim(s) 62-65 and 69, drawn to a methods for the treatment or prevention of a disease with vertebrate Delta.

Group V, claim(s) 66, 67, and 72, drawn to a method for the treatment or prevention of disease via gene therapy.

Group VI, claim(s) 68 , drawn to a method for the treatment or prevention of disease with the antibody.

Group VII, claim(s) 73, drawn to a method for diagnosing disease via Notch:Delta binding.

Group VIII, claim(s) 74, drawn to method for diagnosing disease via Delta levels.

The inventions listed as Groups I-VIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Delta.

Group I is independent of Group II, IV, or VII because while the protein is used to make the antibody or in the methods, the protein can be used in either the methods or in making the antibody. Therefore, these Groups do not relate to a single inventive concept under PCT Rule 13.1.

Group I and III are independent because while the Delta protein can be made recombinantly from the DNA, this protein can also be made recombinantly. Therefore, these Groups do not relate to a single inventive concept under PCT Rule 13.1.

Group I is independent of Groups V, VI, and VIII because the protein is not used in any of these methods. Therefore, these Groups do not relate to a single inventive concept under PCT Rule 13.1.

Group II is independent of Group III because while the DNA is used to make the protein necessary in making the antibody,theseproducts are unrelated in composition and activity. Therefore, these Groups do not relate to a single inventive concept under PCT Rule 13.1.

Group II is independent of Groups IV, V, VII, and VIII because the antibody is not used in any of these methods. Therefore, these Groups do not relate to a single inventive concept under PCT Rule 13.1.

Group II is independent of Group VI because while the antibody is used in the method, it can also be used in isolating Delta. Therefore, these Groups do not relate to a single inventive concept under PCT Rule 13.1.

Group III is independent of Groups IV, VI, VII, and VIII because the DNA is not used in any of these methods. Therefore, these Groups do not relate to a single inventive concept under PCT Rule 13.1.

Group III is independent of Group V because while the DNA is used in the method, it can also be used to make the Delta protein recombinantly. Therefore, these Groups do not relate to a single inventive concept under PCT Rule 13.1.

Groups IV-VIII are independent one from the other because each method uses different products and methods steps to achieve different end results. Therefore, these Groups do not relate to a single inventive concept under PCT Rule 13.1.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/11178

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P ------ Y, P	HENRIQUE et al. Expression of a Delta homologue in prospective neurons in the chick. Letters to Nature. 29 June 1995, Vol. 375, pages 787-790, see entire document.	1, 3, 4, 11-13, 23 ---------- 2, 5, 6-10, 14-22, 24-61, 70, 71, 75-98

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